UNICORN® OS

version 1.10

User Manual

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Edition AA

Amersham
Biosciences

Important user information

Reading this entire manual is recommended for full understanding of the use of this product.

Should you have any comments on this manual, we will be pleased to receive them at:

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Preface

About this manual

This manual provides reference to UNICORN® for Oligo Synthesis version 1.10, referred to as UNICORN OS, from Amersham Biosciences AB. UNICORN OS is a complete package for control and supervision of oligonucleotide synthesis systems, suitable for use with Amersham Biosciences OligoPilot II and OligoProcess. All functionality associated with UNICORN OS consists of software which runs on an IBM-compatible PC under the OS/2 operating environment, and hardware for interfacing the controlling PC to the synthesis module.

The control platform and functions within UNICORN OS are essentially the same as for UNICORN version 2.10, the latter developed by Amersham Biosciences for chromatographic separations. UNICORN OS is supplied with system strategies and templates for oligonucleotide synthesis, although it is possible to perform chromatographic separations associated with UNICORN by obtaining the appropriate strategies and templates from Amersham Biosciences.

In the writing of this manual, those functions that are directly relevant for UNICORN OS are described. Functions that are more relevant to chromatographic separations, such as Scouting, BufferPrep and some evaluation functions are not discussed in this manual.

This manual is organized into 13 chapters and 6 appendices:

Introductory material	1. Introduction 2. UNICORN OS concepts 3. Login and file handling
Methods and runs	4. Creating methods from method templates 5. Creating and editing methods 6. Performing a run
Evaluation	7. Presenting results 8. Evaluating results
System management	9. Security features 10. Network setup 11. Installation 12. Administration 13. System settings
Appendices	A. Technical specifications B. Strategy for Oligo Synthesis C. Evaluation functions and instructions D. Feedback tuning E. File Organization F. Troubleshooting

Assumptions

Two broad assumptions are made in this manual:

1. You should be familiar with the oligonucleotide synthesis systems in your installation. Refer to the appropriate system user manuals for details.

2. You should be familiar with the general principles of using IBM’s OS/2 operating system on your PC. Although UNICORN OS is a self-contained program package and does not require any direct interaction by the user with OS/2, the user interface principles follow the conventions set by the OS/2 operating environment.

Typographical conventions

Menu commands and dialogue box prompts are identified in the text by a **Helvetica** typeface. A colon separates menu levels: thus **File:Open** refers to the **Open** command in the **File** menu.

A `typewriter-like` typeface is used for instructions as they appear in the text editor for methods and evaluation procedures. These are normally entered automatically by UNICORN OS.

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Introductory material

Methods and Runs

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Appendices

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1. Introduction

UNICORN OS is an adapted version of the UNICORN control system which allows real-time control of oligonucleotide synthesis systems from a personal computer. The package operates together with OligoPilot II and OligoProcess from Amersham Biosciences UNICORN OS runs under the operating system OS/2 Warp version 3.0 or higher from IBM.

Functional features of UNICORN OS include:

- One PC may control up to 4 synthesis (and/or chromatographic separation) systems directly.
- Network support allows any number of systems to be run from one PC.
- Method templates, providing method frameworks for most common applications, eliminate the need to program methods from scratch.
- Modular methods are defined in the method templates, reflecting the separate steps in a synthesis process, e.g. detritylation, coupling, oxidation/thiolation, capping and wash/purge steps.
- Dynamic graphical overview of active runs.
- User-definable alarm and warning limits for monitor signals.
- Batch operation and process documentation in accordance with the requirements of Good Manufacturing Practice (GMP) and Good Laboratory Practice (GLP).

In addition, UNICORN OS offers a comprehensive security system:

- Password control for all users, with access authorization for other users' method and result files.
- Customized definition of access control levels.
- Audit trail for system operation.

Note: UNICORN OS must be correctly installed for stand-alone or network operation before the software can be used. Network considerations, software installation and administration of system and user definitions are described in Chapters 10, 11 and 12 of this User Manual.

2. UNICORN OS concepts

This chapter introduces the basic concepts that are needed to use UNICORN OS. For a description of general concepts of how to work with the OS/2 operating system, see your OS/2 system documentation.

Material in this chapter is divided into 7 sections, dealing with:

- ••UNICORN OS user interface
- ••Files and directories
- ••Methods and method structure
- ••System control
- ••Evaluation
- ••Network considerations
- ••Security and administration

2.1 UNICORN OS user interface

2.1.1 UNICORN OS control software

UNICORN OS runs under the OS/2 operating system, and provides facilities for method-controlled operation of oligonucleotide synthesis systems as well as real-time monitoring and subsequent evaluation of the synthesis process.

Strategies

Part of UNICORN OS software (referred to as the *strategy*) is system specific. The strategy defines what is available in method and manual instructions, system settings, run data, curves and method templates. Most of this manual describes the user interface in UNICORN OS independent of the strategy. Strategy-dependent instructions are listed in Appendix B.

2.1.2 Stand-alone and network installations

UNICORN OS may be installed either on a stand-alone computer or in a network.

- In a stand-alone installation, up to four oligonucleotide synthesis (and/or chromatographic separation) systems may be physically connected to and controlled from the computer where UNICORN OS is installed.

- In a network installation, each oligonucleotide synthesis system is physically connected to one computer in the network, but may be controlled from any computer in the network on which the software is installed. A computer to which oligonucleotide synthesis systems are physically connected is referred to as a *local station*. Other computers in a network installation are called *remote stations*.

Note: It is not necessary to install UNICORN OS for network control if you only want to be able to save method and result files on a server disk. Just define a home directory on the server disk if the computer itself is connected to a network (see Section 11.4.1).

2.1.3 Software modules

UNICORN OS control software consists of four integrated modules:

- The **Main menu**, with functions for file handling and administrative routines such as definition of available oligonucleotide synthesis systems and maintenance of user profiles.

- The **Method editor**, where methods for pre-programmed control of oligonucleotide synthesis systems are created and edited.

- The **System control** module, which permits manual or method-based control of oligonucleotide synthesis systems and on-line monitoring of synthesis processes. There may be up to four independent System control modules on one computer, for controlling up to four separate systems.

- The **Evaluation** module, for viewing and presenting stored results from synthesis processes.

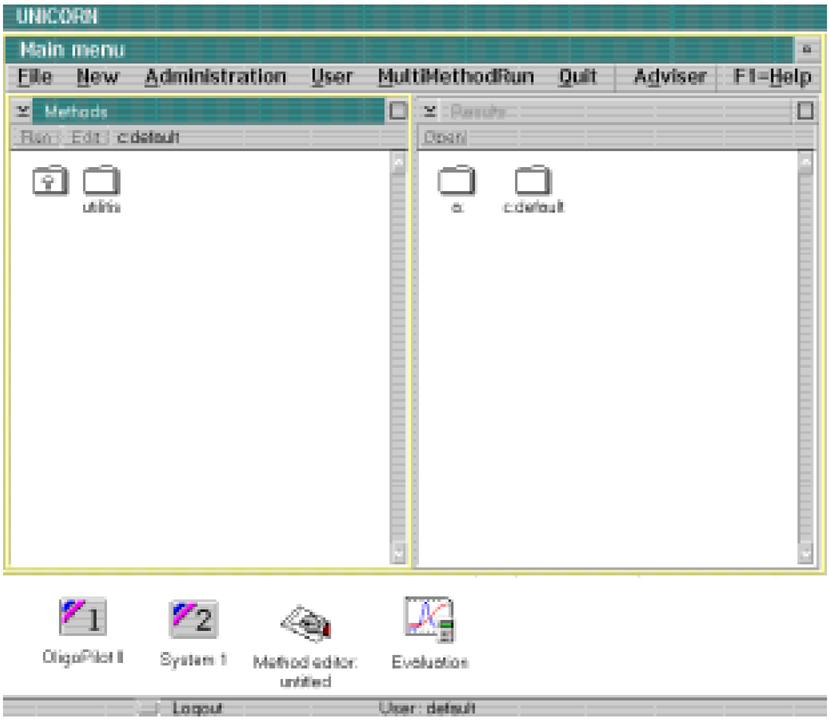


Figure 2-1. *UNICORN OS desktop with the Main menu shown as a window and the other modules as icons.*

These modules are represented by either windows or icons on UNICORN OS desktop (see Figure 2-1). UNICORN OS desktop itself occupies the entire screen. According to how the software is installed, you may be able to minimize UNICORN OS to allow access to other programs on the OS/2 desktop.

2.1.4 OS/2 terminology

This section explains some of the basic terms relating to the OS/2 user interface. Refer to your OS/2 documentation for a complete description.

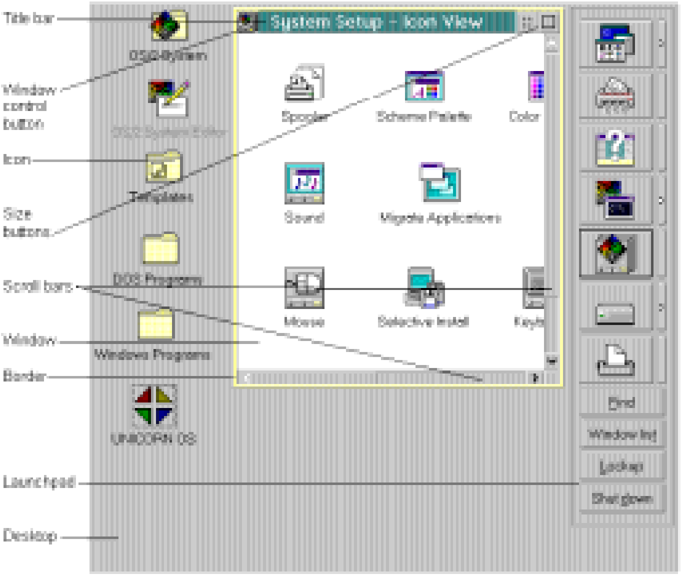


Figure 2-2. Main components of the OS/2 user interface.

- The *desktop* is the area of the screen in which windows can be opened. UNICORN OS has its own desktop which may occupy all or part of the screen.

- An *icon* is a symbol on the desktop or in a window representing an object in the interface (usually a file or program). In general, double-click on an icon with the left mouse button to open the icon to a window. Double-clicking on an icon on the OS/2 desktop generally starts the program associated with the icon. Icons on UNICORN OS desktop represent modules in UNICORN OS software, and double-clicking on a module icon opens the window for the module.

- A *window* is an active area of the desktop used by a program or an open folder displaying its contents. There may be several windows open at the same time on the OS/2 desktop, but only one is active at any one time. The active window is generally identified by the colour of the title bar and border. In UNICORN OS the **Autominimize** setting under **Preferences** in the Main menu determines whether several windows can be open at the same time. The recommended setting with **Autominimize** selected reduces the active window to the corresponding icon if another module is opened, reducing potential confusion on UNICORN OS desktop.

- Drag the window *border* to change the size of the window.

- Use the *scroll bars* to scroll the window contents. Scroll bars are only shown when the window contents do not fit in the current window size.

- Each window has a *title bar* which identifies the program or folder (in OS/2) or module (in UNICORN OS) associated with the window. In UNICORN OS, the title bar for the System control window can be set to different colours according to the identity of the oligonucleotide synthesis system connected to the window (see Section 14.1).

□ **Maximize**

Restore

- At the right hand end of the title bar are buttons for quickly adjusting the size of the window. Clicking on the **Maximize** button enlarges the window to fill the desktop. At the same time, the button changes to the **Restore** button, which restores the window to its previous size. If the window is not maximized, you can drag the window borders to adjust the size of the window.

  Note: Depending on how UNICORN OS has been installed, there may also be a minimize button to reduce the window to an icon.

- At the left hand end of the title bar on OS/2 windows is a button for window control functions. This button is not used in UNICORN OS windows.

- The *Launchpad* is located on the OS/2 workspace and allows applications to be launched with a single click of the mouse button. It is possible to link the UNICORN OS icon to the Launchpad.

- Application program windows (including UNICORN OS) have a *menu bar* with menu commands (see Figure 2-1). Click on a menu item or press <Alt> followed by the underlined letter in the item to open the menu. Click on a command in the menu or press the underlined letter in the command to activate the command.

2.1.5 On-line help

F1=Help

Help

A comprehensive on-line help utility is included in UNICORN OS software. Choosing **Help** from the menu or pressing function key F1 provides an entry to the help texts for the currently active module. **Contents**, **Search** and **Index** functions help the user to find information for all modules, regardless of which module is currently active. Most dialogue boxes have a **Help** button for help on how to use the currently open dialogue box.

In the instructions boxes for method instructions, procedure instructions and system settings, pressing F1 when an instruction is highlighted will display an information box with short help on the function and usage of the selected instruction.

2.2 Files and directories

UNICORN OS Main menu interface divides user files into two categories, for methods and results (see Figure 2-1). Only directories to which the current user has access are shown in the Main menu panels.

Files may be displayed in icon view or details view in the Main menu panel (see Chapter 3 for more details).

2.2.1 Method files

Method files contain instructions for controlling a run and are shown in the **Methods** panel of the Main menu.

2.2.2 Result files

Result files are created by UNICORN OS when a synthesis process is run and contain:

- Settings for the synthesis run (method variables and questions)
- File name, sequence and description for the sequence
- Efficiency values for each coupling stage

2.3 Methods

Oligonucleotide synthesis runs are programmed as *methods* in UNICORN OS. UNICORN OS is supplied with method templates which enable most synthesis processes to be performed. The user needs only to insert the desired sequence into the sequence editor and then click on the associated **Create** button to automatically produce a ready-to-run method (see Chapter 4). For more advanced usage, it is possible to edit and create methods for specific synthesis processing needs (see Chapter 5).

2.3.1 Method structure

This section introduces the main components of a method and how they are structured. In most cases, it is sufficient to just run the method after you have provided information about the sequence you want to synthesize (see Section 4.3). More advanced usage of UNICORN OS requires that you understand the principles and concepts of methods.

Blocks

Methods in UNICORN OS are usually divided into blocks. Blocks typically contain the subroutines that control the complete synthesis procedure. A synthesis cycle is generally based on the following order of subroutines:

- ••Detritylation
- ••Detrit wash
- ••Coupling
- ••Oxidation/thiolation
- ••Capping

Method templates supplied with UNICORN OS contain all the blocks that are likely to be used in a specific method. When the desired sequence is created, the blocks needed to build up the method to synthesize the sequence are automatically copied in from the method template. The methods derived from the method templates can be directly used to process the run.

Additionally, the methods are convenient starting points for developing customized methods. Fully adequate customized methods for many applications can be created simply by adjusting the values of method variables (see below). New blocks can also be created in the Text instructions or in the cross reference list of the Sequence page in Run setup.

Method base

Method blocks are written in one of three method bases, which defines the unit for the breakpoints in the block:

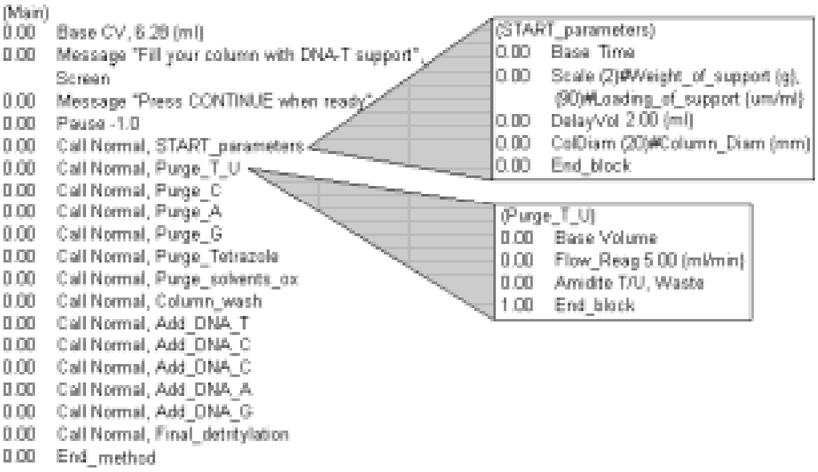
- ••time (min)
- ••volume (ml or l according to the strategy)
- ••column volume (set by the user)

Different blocks in the same method may be written with different method bases: for example a column wash block might be written in terms of column volumes while a purge block might be best expressed in absolute volume.

Note: The term *method base* should not be confused with bases in a sequence or an oligonucleotide.

Instructions
The method is a call to blocks, with each block containing a series of *instructions* or sub-routines (see Figure 2-3). Each instruction is a request for specific operations in the system. A block may also contain other blocks which in turn contain their own series of instructions.

Double click on a block to expand/collapse the view of the instructions.



```
(Main)
0.00    Base CV, 6.28 (ml)
0.00    Message "Fill your column with DNA-T support",
        Screen
0.00    Message "Press CONTINUE when ready"
0.00    Pause -1.0
0.00    Call Normal, START_parameters
0.00    Call Normal, Purge_T_U
0.00    Call Normal, Purge_C
0.00    Call Normal, Purge_A
0.00    Call Normal, Purge_G
0.00    Call Normal, Purge_Tetrazole
0.00    Call Normal, Purge_solvents_ox
0.00    Call Normal, Column_wash
0.00    Call Normal, Add_DNA_T
0.00    Call Normal, Add_DNA_C
0.00    Call Normal, Add_DNA_C
0.00    Call Normal, Add_DNA_A
0.00    Call Normal, Add_DNA_G
0.00    Call Normal, Final_detritylation
0.00    End_method
```

```
(START_parameters)
0.00    Base Time
0.00    Scale (2)#Weight_of_support (g),
         (90)#Loading_of_support (um/ml)
0.00    DelayVol 2.00 (ml)
0.00    ColDiam (20)#Column_Diam (mm)
0.00    End_block
```

```
(Purge_T_U)
0.00    Base Volume
0.00    Flow_Reag 5.00 (ml/min)
0.00    Amidite T/U, Waste
1.00    End_block
```

Figure 2-3. *Relationship between blocks and instructions. The method (left) is written as a series of calls to blocks, each of which consists of instructions for performing one or more specified tasks (right).*

Breakpoints
Each instruction in a method block is issued at a specified *breakpoint* according to the method base. The first instruction in a block is always at breakpoint 0, and all other breakpoints are counted from this point. For example, in the following instructions from a block:

```
0.00    Base Time
0.00    Flow_Reag 5.00 {ml/min}
9.00    Flow_Reag 0.00 {ml/min}
```

At breakpoint 0.00, the reagent flow rate is set to 5.00 ml/min. After nine minutes have elapsed, at the next breakpoint, the flow rate of the reagent will be set at 0.00 ml/min, i.e. no flow at all.

Method variables

Breakpoint values and instruction parameters may be defined as *variables*. This is a powerful facility for constructing a method which contains default parameter values. These default values may then easily be changed either to create variants of the same method or to adjust the parameter values at the start of a run (see Section 4.4).

Using variables makes it easy to adapt a method to a particular oligonucleotide synthesis run. For example, in the block below, the values of the start parameters variables can be seen:

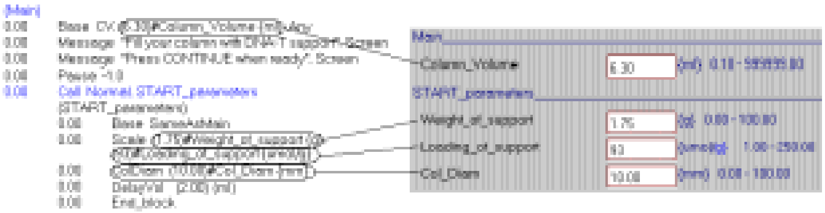
```
(START parameters)
0.00  Base SameAsMain
0.00  Scale (2)#Weight_of_support {g},
      (90)#Loading_of_support {umol/g}
0.00  DelayVol 2.00 {ml}
0.00  ColDiam (20)#Col_Diam {mm}
4.00  End_block
```

The variables are expressed:

(variable value)#Variable_type{ variable units }.

In the block above, it is possible to see that the variable values have been set at 2 g weight of support, 90 µmol/g loading of support and a 20 mm column diameter.

By using variables, a method may be displayed either in detail as text instructions or in a condensed form as variable values in Run setup mode. This is illustrated in Figure 2-4. The Run setup mode is displayed when the method is run, allowing variable values to be set at the beginning of the run.



```
(Main)
0.00    Base CV (6.30)#Column_Volume {ml} Any
0.00    Message "Fill your column with DNA-T support" Screen
0.00    Message "Press CONTINUE when ready". Screen
0.00    Pause -1.0
0.00    Call Normal START_parameters
        (START_parameters)
        0.00    Base SameAsMain
        0.00    Scale (1.75)#Weight_of_support {g}
                (93)#Loading_of_support {umol/g}
        0.00    ColDiam (10.00)#Col_Diam {mm}
        0.00    DelayVol (2.00) {ml}
        0.00    End_block
```

Main

Column_Volume 6.30 {ml} 0.10 - 999999.00

START_parameters

Weight_of_support 1.75 {g} 0.00 - 100.00

Loading_of_support 93 {umol/g} 1.00 - 250.00

Col_Diam 10.00 {mm} 0.00 - 100.00

Figure 2-4. *Relationship between variables in text instructions and in the Variables page of run set-up.*

2.4 System control

2.4.1 Control facilities

The System control module allows independent control of up to four oligonucleotide synthesis systems from one computer, with continuous real-time monitoring of the synthesis process. The run status can be displayed as:

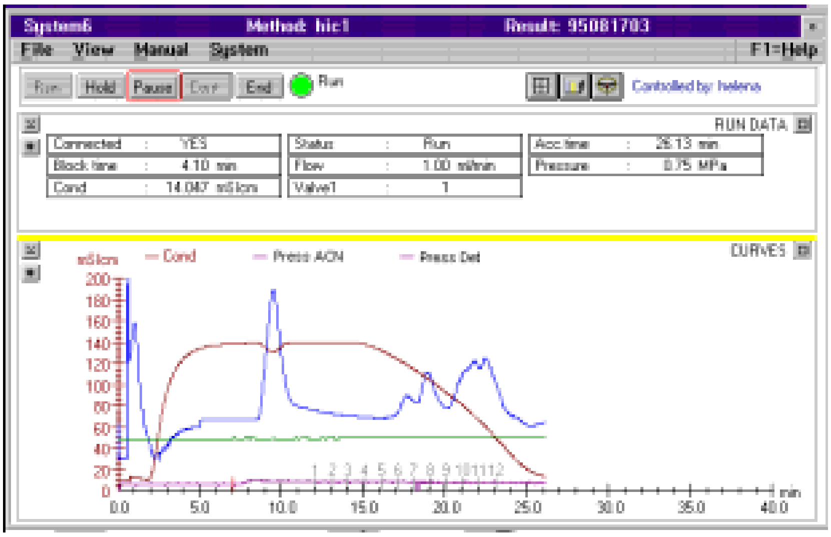
••numerical display of run data from selected monitors

••graphical display of curves from monitors

••a flow scheme showing the current flow path in the system

••a logbook recording the control events in the run.

Systems can be controlled either manually with interactive commands or through pre-programmed methods.



Connected : YES
Block time : 4.10 min
Cond : 14.047 mS/cm
Status : Run
Flow : 1.00 ml/min
Valve1 : 1
Acc time : 26.13 min
Pressure : 0.75 MPa

Figure 2-5. The System control window with run data and curves displayed.

2.4.2 System connections

To control a synthesis process, the operator establishes a *connection* between the computer and the oligonucleotide synthesis system in one of the System control windows on UNICORN OS desktop. Two kind of connections may be established:

- •*Control mode connections* which permit full control of the connected system.

- •*View mode connections* from which the progress of the synthesis can be monitored but the system cannot be controlled.

Each oligonucleotide synthesis system may have only one control mode connection but may have several view mode connections. In a network installation, the same or different users may establish simultaneous view mode connections to one system on different computers. This allows a running process to be monitored from several locations at the same time.

The actual control of running processes is handled by the local computer. A user may disconnect a control mode connection without affecting the running process.

2.5 Evaluation

The Evaluation module provides extensive facilities for presentation and evaluation of synthesis results. Essential features of evaluation include:

- •*Trityl data*. This is stored in the result file and can be printed in a report as a table

- •*Curve manipulation*. A wide range of operations can be performed on curves, such as addition and subtraction of two curves, differentiation, normalization and scaling. The original raw data curves are always kept unmodified in the result file.

- •*Curve comparisons*. Curves from different result files can easily be compared in the Evaluation module.

- •*Evaluation procedures.* Operations performed in the Evaluation module can be recorded as an evaluation procedure and repeated for other result files with a single menu command. Evaluation procedures may be executed either automatically on completion of a method run or interactively from within the Evaluation module.

- *Reports.* Comprehensive reports of the evaluation results can be generated for hard-copy documentation of the synthesis process. Generation and printing of reports may be included as an operation in an evaluation procedure to automate process evaluation and documentation.

The Evaluation module is described in detail in Chapters 7 and 8.

2.6 Network considerations

Figure 2-6 illustrates how a networked UNICORN OS installation can be organized. There are two kinds of PC where UNICORN OS software is installed:

- A *local station* is a PC to which oligonucleotide synthesis systems are physically connected.

- A *remote station* is a PC to which no systems are physically connected, but which can control systems over a network link.

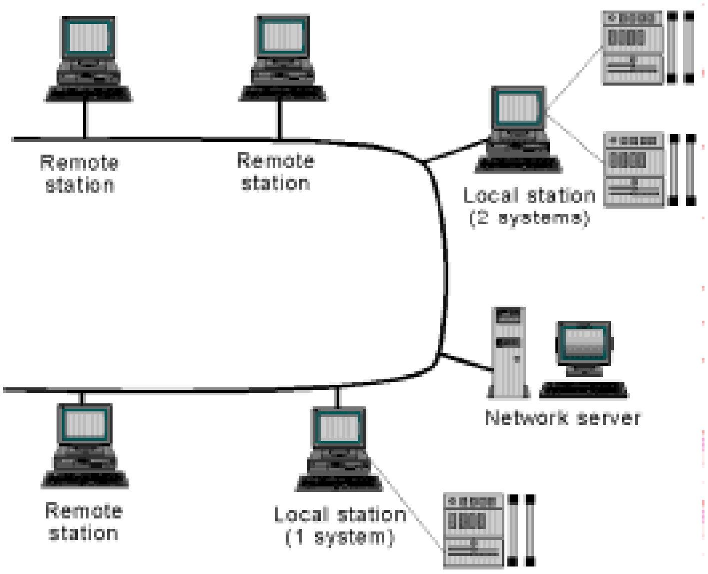


Figure 2-6. *A network installation with 3 oligonucleotide synthesis systems and 5 stations (3 remote and 2 local). The systems can be controlled from any computer except the network server.*

Oligonucleotide synthesis systems in the network can be controlled from either remote or local stations. A local station in a network can also be used as a remote station to control other systems.

Note: If a run is being controlled from a remote station and a network communication error occurs, the run will continue under the control of the local station. Results will be saved in the FAILED directory on the local station (see Section 6.4). A control mode connection can be established on the local station to control the run.

For an oligonucleotide synthesis system to be accessible in the network, the local station must be switched on and logged in to the network. UNICORN OS user interface does not however need to be started on the local station. System control from a remote station is managed through network level routines which are started in **startup.cmd** on the local computer (see Figure 2-7).

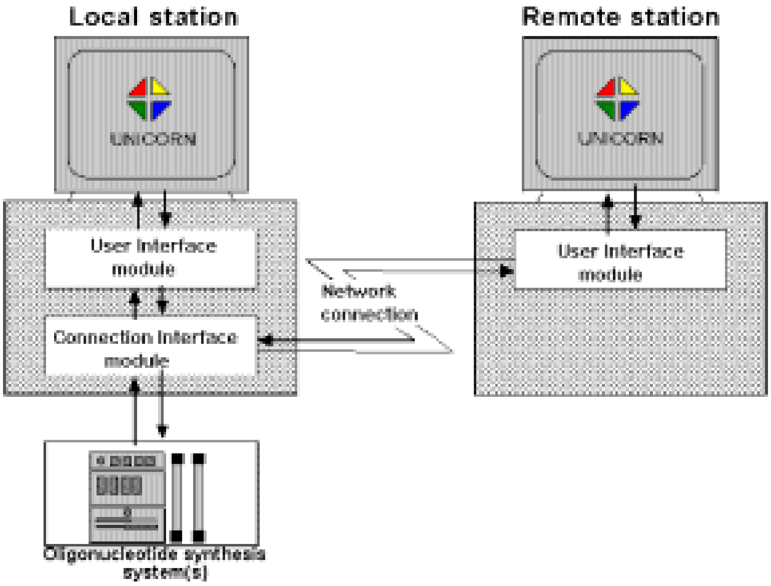


Figure 2-7. *The connection interface module in a local station is started automatically and runs separately from the user interface module. This allows systems connected to the local station to be controlled from a remote station without running the user interface module on the local station.*

A local station can be used to control the oligonucleotide synthesis systems directly connected to the PC without logging in to the network. Method and result files stored on network drives will of course not be accessible. For runs performed in this stand-alone mode where the result file is directed to a network drive, the results will be saved in the FAILED directory on the local station (see Section 6.4).

2.7 Security

Security features in UNICORN OS include:

- *Access security*. Use of UNICORN OS is restricted to authorized users. Each user is assigned an access level which defines the functions that the user is permitted to use.

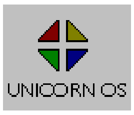
- *Connection security*. Running systems may only be controlled from one connection. Systems may be locked with a password to prevent other users from changing run parameters.

- *Data security*. Result files can be saved automatically at pre-set intervals during a run to minimize data loss in the event of system failure. In a network installation, results are saved on the local station if network communication fails.

Security features are discussed in more detail in Chapter 9. Network and administrative aspects are discussed in Chapters 10 and 11 respectively.

3. Login and file handling

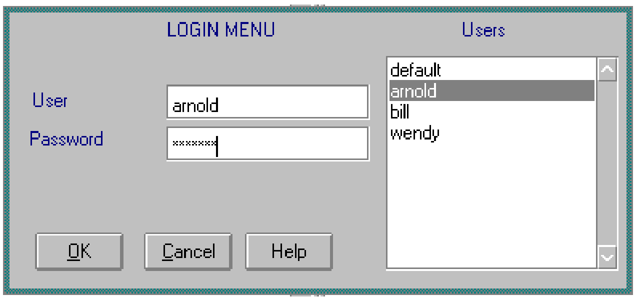
3.1 Logging in



Desktop icon

To start UNICORN OS, double-click on the UNICORN OS icon on the OS/2 desktop. The program takes a little while to start.

If UNICORN OS is already started and the previous user has logged out, click on the **Login** button at the bottom of the UNICORN OS desktop.



LOGIN MENU

User arnold

Password *******

Users
default
arnold
bill
wendy

Figure 3-1. *The Login Menu dialogue box.*

Click on your username in the list or type the name directly in **User** field. Type your password in the **Password** field and then click on the **OK** button to log in.

If you cannot remember your password, you cannot log in to UNICORN OS. Ask your system administrator or other user with sufficient authorization to give you a new password.

Press the **Cancel** button to abandon the log-in attempt. If you press **Cancel** during initial start-up of UNICORN OS, the system returns to the OS/2 desktop.

Network installations
In a network installation, you must log in to the network before starting UNICORN OS.

If you want to control a system from the local computer without logging in to the network, press **Enter** when the startup.cmd window appears. Do not just minimize or close the startup.cmd window.

You can log in to UNICORN OS on any computer in the network, provided that UNICORN OS software is installed on that computer. You can log in with the same username and password on any number of computers simultaneously. All computers can connect to and display a running system, but a given system can only be actively controlled from one computer. Multiple log-ins with the same username are treated internally as separate users for the purpose of System control.

Note: Do not confuse network log-in with UNICORN OS log-in. You log in to the *network* to gain access to network resources (shared drives, printers and other networked equipment). You log in to UNICORN OS to gain access to the oligonucleotide synthesis systems that are installed in the network. The username and password for logging in to the network are entirely independent of the those for logging in to UNICORN OS.

3.2 UNICORN OS desktop

3.2.1 Icons and windows

Each of the four modules in UNICORN OS is represented by an icon or an open window on UNICORN OS desktop (see Figure 3-2). On start-up, the Main menu is presented as a window and the other modules as icons. There may be several icons for System control, according to how the program was installed. There is however only one icon for the Main menu, Method editor and Evaluation module.

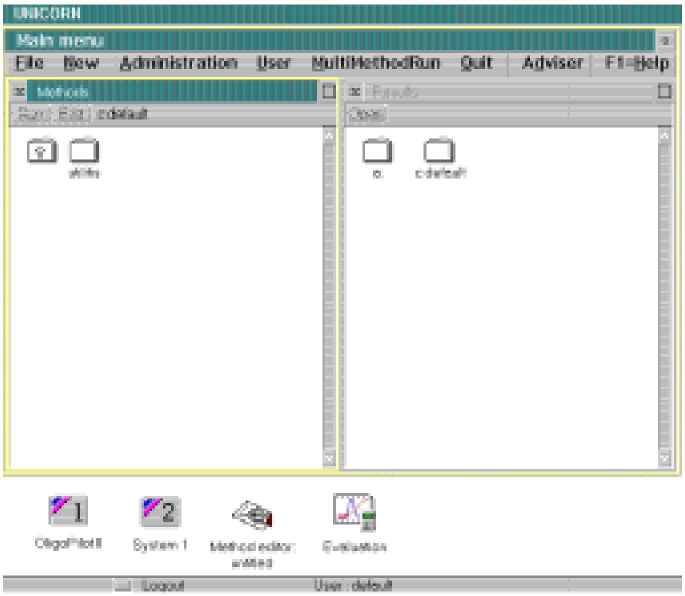


Figure 3-2. *UNICORN OS desktop with the Main menu active.*

To expand a module to a window, double-click on the corresponding icon. If **User:Autominimize** is checked in the Main menu, only one window can be expanded at once, and expanding one icon to a window automatically minimizes the previously active window. If **User:Autominimize** is not checked, any number of windows can be expanded at the same time. You can drag window borders to adjust the size of module windows (and of panels and windows within the module windows).

To minimize a window to an icon, click on the **Minimize** button at the right-hand end of the window title-bar. Depending on how UNICORN OS is installed (see Section 11.4), you may also be able to minimize UNICORN OS window to an icon on the OS/2 desktop.

Note: Minimizing a module window to an icon does not close the module. Once opened, UNICORN OS modules remain open until you quit the program. A minimized System control icon may thus be actively in control of a running process.

3.2.2 UNICORN OS Main menu panels

The two Main menu panels display method and result files respectively. Use the panels as follows:

- ••Click anywhere in the panel (outside an icon or file name) with the right mouse button to pull down a menu for setting the panel presentation.
- ••Click on an icon or file name with the right mouse button to pull down a menu for opening, copying, moving, renaming or deleting the file. Method files can also be opened for either System control (**Run**) or editing (**Edit**) from this menu.

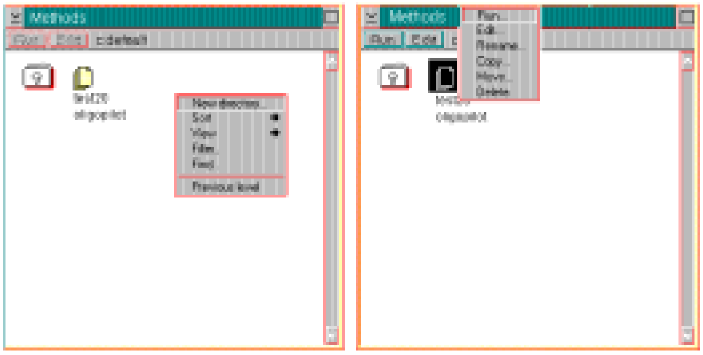


Figure 3-3. *The Methods panel showing pull-down menus for panel presentation (left) and file management (right)*

- Alternatively, double-click on an icon to open the file. For method files, this opens the file in the Method editor. For result files, this opens the file in the Evaluation module.

- Select a method file and click on **Run** or **Edit** to run or edit the method respectively.

To change the size of a panel, drag the panel border. Click on the **Maximize** button at the right-hand end of the panel title bar to toggle panel size between normal and maximized. (Maximizing a panel will hide but not close the other panel.)

3.2.3 Presenting files

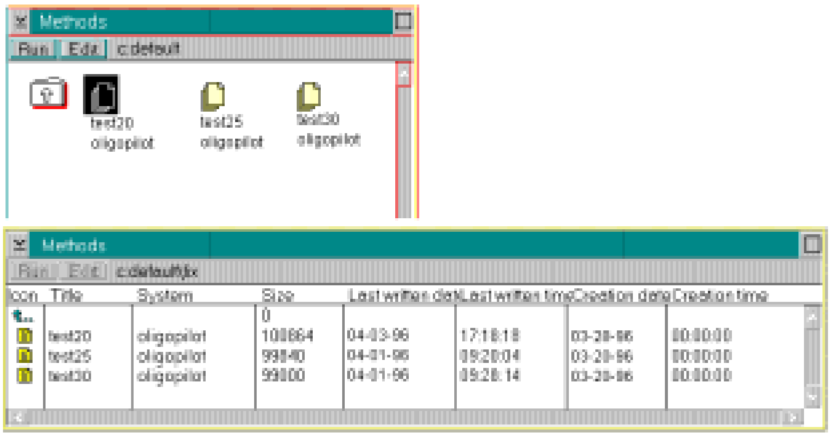
The way files are presented in the panel can be set from the **File** menu or from the pull-down menu that appears when you click with the right mouse button in the panel. Presentation options are

- view mode (icons or detail display)
- sorting order
- filter (for displaying only a chosen set of objects, e.g. methods for one system)

View

You can display the contents of the panels either as graphical icons (**View:Icons**) or as a details list (**View:Details**). The details list includes a small icon identifying the type of object, file size, last written date and time and creation date and time.

To change the view mode, choose **View** from the pull-down panel menu or from the **File** menu, and choose the appropriate mode from the sub-menu.



Icon	Title	System	Size	Last written dat	Last written time	Creation date	Creation time
↰..			0				
▨	test20	oligopilot	100864	04-03-96	17:18:18	03-20-96	00:00:00
▨	test25	oligopilot	99840	04-01-96	09:20:04	03-20-96	00:00:00
▨	test30	oligopilot	99000	04-01-96	09:20:14	03-20-96	00:00:00

Figure 3-4. *Icon and detail display modes illustrated for the methods panel.*

Sort
Files can be sorted in the panel according to one of

••name (alphabetical order)
••type (directories and files)
••size (smallest files first)
••creation date (oldest files first)
••last saved date (least recently modified files first)

To change the sorting order, choose **Sort** from the pull-down panel menu or from the **File** menu, and choose the appropriate sorting order from the sub-menu. Changing the sorting order affects only the currently active panel.

Filter
To restrict the files displayed according to file name or the system with which they are associated, choose **Filter** from the pull-down panel menu or from the **File** menu. Mark the system(s) for which you want to display files, and enter a file name specification if required. Click on **OK** to activate the filter. The filter affects the display in both panels.

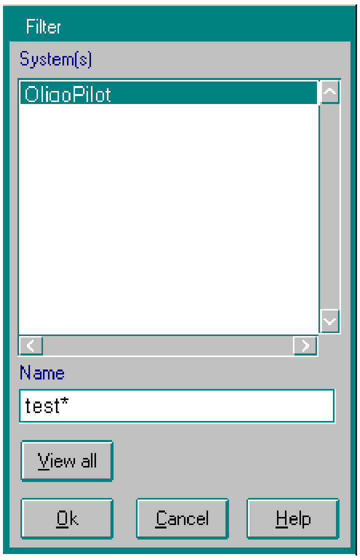


Figure 3-5. *The Filter dialogue box.*

You can use standard OS/2 wildcard characters in the file name specification (* stands for any number of characters, ? for any single character). For example:

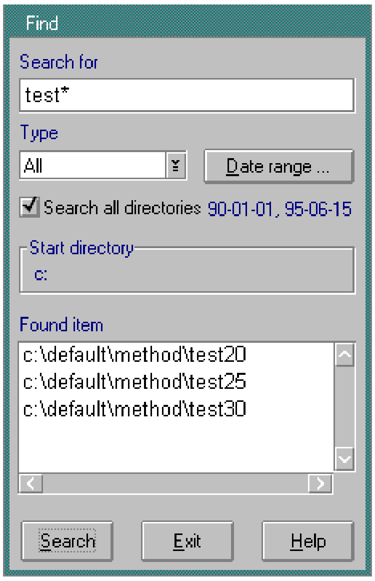
test	will display only files named test
test*	will display all files with names beginning with test
*test	will display all files with names ending with test
?test	will display only 5-character names ending with test

If a filter is active, this is indicated in the title bar of the panel (e.g. **Results : filtered igf***). To display all files, choose **Filter** and click on **View All**.

3.2.4 Finding files

To find a file:

1. Choose **Find** from the pull-down panel menu or from the **File** menu. Enter a file name specification in the **Search for** field.



Find

Search for: test*

Type: All

☑ Search all directories 90-01-01, 95-06-15

Start directory: c:

Found item:

```
c:\default\method\test20
c:\default\method\test25
c:\default\method\test30
```

***Figure 3-6.** The Find dialogue box.*

You can use standard OS/2 wildcard characters in the file name specification (see above).

2. You can restrict the search further if required:

••Choose file type from the pull-down menu for **Type** (**All**, **Directory**, **Method** or **Sequence** for the methods panel; **All**, **Directory**, **Result** or **Sequence/Scouting** for the results panel).

••Click on **Date Range** for files last written between specified dates.

••Check **Search all directories** to search through all the directories to which you have access. If **Search all directories** is not checked, the search will be restricted to the current directory and sub-directories below.

3. Click on **Search** when you have entered all parameters. The result of the search is shown in the **Found item** box.

4. Double-click on a file in this list to return to the Main menu with the selected file highlighted in the appropriate panel. If you click on **Exit** (with or without selecting a file), you will return to the Main menu with the panel display unchanged.

3.2.5 Copying, moving and renaming files

You can copy, move or rename files and directories using commands from the pull-down file menu or from the Main menu bar. If you copy or move directories, all files within the directories will also be copied or moved.

1. Select one or more files in the Methods or Results panel of the Main menu. To select multiple files, hold down the <Ctrl> key while you click on the file names or icons. (**Rename** is not available if you select multiple files.)

2. Click with the right mouse button on any file icon and choose the appropriate command from the pull-down file menu, or choose the command from the **File** menu in the Main menu bar.

3. For **Copy** and **Move**, choose the directory to which you want to copy the file and click on **OK**. For **Rename**, enter a new name for the file or directory.

Notes*:* To copy or move a method or result file to a diskette, choose the **a:** folder as the target directory. A file that has been stored on a diskette cannot be opened directly from the diskette. To use a file from a diskette, first copy the file back to an appropriate folder on the hard disk.

To copy a file within the same directory, use the **File:Save as** command in the appropriate module after opening the file. The copy must have a different name from the original.

You cannot copy or move files between the Methods and Results panels of the Main menu.

Explicit authorization is required to copy or move files (see Chapter 9).

3.2.6 Deleting files

To delete a file or directory:

1. Select the item(s) to be deleted in the Methods or Results panel of the Main menu. To select multiple files, hold down the <Ctrl> key while you click on the file names or icons.

2. Click with the right mouse button on any file icon and choose **Delete** from the pull-down file menu, or choose **Delete** from the **File** menu in the Main menu bar.

3. Confirm the deletion in the dialogue box.

Notes: Home directories cannot be deleted by this method (see Chapter 12).

Explicit authorization is required to delete files (see Chapter 12).

A file that has been deleted cannot be recovered except by restoring a back-up copy or using a third-party utility for recovering lost files.

3.2.7 Backup security

To protect important data against accidental deletion or loss in the event of hard disk failure, backup copies should be taken at regular intervals. To make back-up copies, you can either copy files to diskette using the **File:Copy** command in UNICORN OS (choose the **a:** folder as the target diskette for the copy operation), or use OS/2 backup functions from the OS/2 Workplace Shell. A tape streamer is however recommended for efficient backup handling from OS/2. If you are working in a network, you can copy files to the network server (make sure that the network administrator runs server back-ups regularly). Backup copies should be stored in a safe place, physically separated from the PC.

The responsibility for making backup copies rests entirely with the user. Amersham Biosciences cannot undertake to replace method programs lost as a result of computer failure or other incident.

3.3 Logging out

To log out of UNICORN OS, press function key F12 or click on the **Logout** button at the bottom of UNICORN OS desktop.

Processes that are running when you log out will continue to run, and may be left locked with a locking password or unlocked (see Section 6.5 for more details). Any modules that were active at the time of log-out (e.g. unsaved methods or evaluation sessions) will be re-opened when the same user logs in again.

UNICORN OS will still be open after a user has logged out, and another user may log in. We recommend that you always log out when you leave the computer, to prevent other users from accidentally changing or deleting your files or disturbing your runs.

3.4 Quitting UNICORN OS

To quit UNICORN OS and close the program, choose **File:Quit program** or **Quit:Quit program** from the Main menu. You will be prompted to save any unsaved work in the Method editor or Evaluation module. You can only quit the program from the Main menu.

Note: You can quit UNICORN OS during a run which does not use scouting or a sequence without affecting the run. Do not however shut down OS/2 or turn off the computer while the run is in progress.

Introductory material

Methods and Runs

Evaluation

System management

Appendices

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4. Creating methods from method templates

UNICORN OS is supplied with a set of method templates which are used as the starting point for creating customized methods. These method templates are defined with variables for critical parameters in the synthesis, so that customized methods can be created for most purposes simply by setting appropriate values for the method variables. This chapter describes how to create and edit methods at this level. See Chapter 5 for a description of advanced method editing facilities.

Briefly, the steps in creating a method by editing method variables are as follows:

1. Choose **New:Method** in the Main menu or the Method editor and select a system and template.

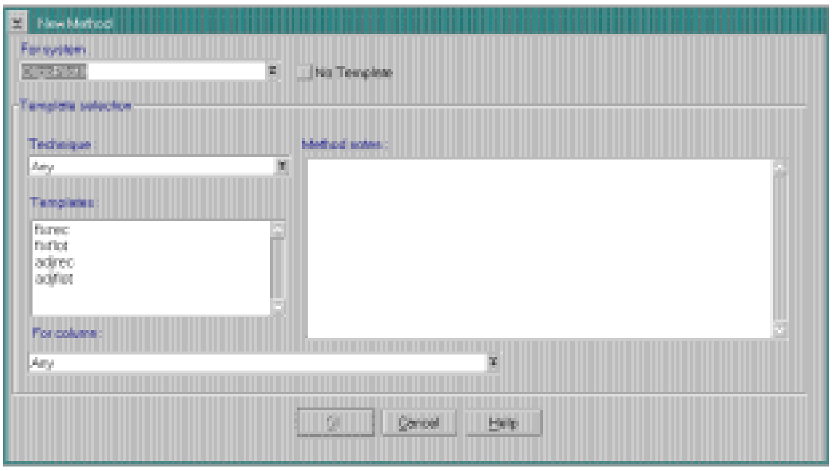
Run setup

2. Choose **View:Run setup** or press the **Run setup** button.
3. Open the Sequence page and enter the desired sequence and create the method. Click on the **Save As** button in the Sequence page if the sequence should be saved for future use.
4. Enter the method variables.
5. Read the method notes.
6. Save the method.
7. Run the method

4.1 Creating a new method

New method

To create a new method, click on the **New Method** toolbar button or choose the **File:New:Method** menu command in the Method editor, or choose **File:New:Method** or **New:Method** in the Main menu. These alternatives are equivalent. When you choose the command from the Main menu, the Method editor is opened automatically.



Technique: Any

For column: Any

__Figure 4-1.__ The New method dialogue box.

1. Choose the system for which the method is intended.
   The instructions available for a given system are determined by the system strategy. A method developed for one system may not be valid on another.

2. Select **Any** as the technique.

3. A list of ready-to-run method templates is displayed for the selected technique. Available templates are determined by the system strategy. Select one of these templates to create customized methods either by adjusting variable values (see Chapter 4) or changing method instructions. For your first run, you are recommended to select the method template, **fixrec**.

   Click on a template to display information about the particular template in the **Method notes** field.

4. Choose **Any** in the **Column** pull-down list.

5. Click on **OK** once you have made your selections. The method template will now be opened as an untitled method.

4.2 Saving and running a test sequence method

All newly created templates already contain a partially built method for a pre-defined 20 base sequence, which is:

5´ ATA CCG ATT AAG CGA AGT TT 3´

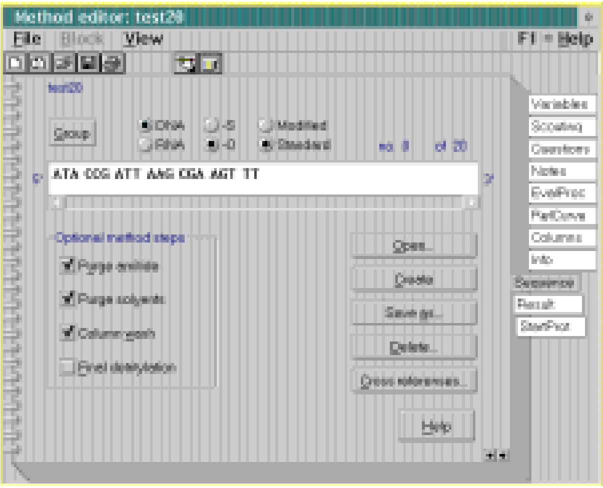
This sequence can be viewed in the sequence editor in the Sequence page of Run setup, or the blocks for the sequence as blocks in the Text instruction panel. This partial method has two main uses:

- ••Used specifically with the **fixrec** method template, you can save the method and directly perform a test run of UNICORN OS to synthesize the sequence (see below)
- ••Used with any method template, you can replace the supplied sequence with a sequence of your own choice and then generate a ready-to-run method for that sequence (see Section 4.3).

To use the 20 base sequence for a test run of the instrument:

Run setup

1. Create a new method according to Section 4.1.
2. Click on the **Run set-up** button on the toolbar or select **View:Run setup**.
3. Select the Sequence page to display the pre-defined sequence in the sequence editor field.



Method editor: test20

test20

● DNA ○ RNA ○ -S ● -O ○ Modified ● Standard no. 0 of 20

5' ATA CCG ATT AAG CGA AGT TT 3'

Optional method steps

☑ Purge amidite

☑ Purge solvents

☑ Column wash

☐ Final detritylation

Figure 4-2. Sequence page in Run setup containing the 20 base sequence pre-defined in the method template.

3. Click on the **Create** button (see Section 4.3), which fully generates the method and inserts default variable values. The **Save As** dialogue is then displayed. Save the method with the name **test20.**

   The method must be saved before you can make a run. The **test20** method will be displayed in the **Methods** box of the Main menu.

4. To run the **test20** method, follow the instructions detailed in Section 4.7 and Chapter 6. You can change the method variables prior to the commencement of the run.

4.3 Creating a sequence and method

As described in Section 4.2, all of the method templates contain a partially built method for a pre-defined 20 base sequence. By replacing the pre-defined sequence with a sequence of your choice, you can quickly and easily create a ready-to-run method.

1. Select a method template in the **New Method** dialogue box, as described in Section 4.1.

2. Click on the **Run set-up** button on the toolbar or select **View:Run setup**.

3. Select the Sequence page to display the pre-defined sequence. Select the pre-defined sequence in the sequence editor and delete it using the <Delete> key on the keyboard.

4. Enter a new sequence, up to a maximum of 200 bases, in the sequence editor in the 5´-3´ direction. Remember that the synthesis always proceeds in the reverse direction, i.e. 3´-5´ direction. By default, a DNA sequence consisting of oxidated bases taken from the standard reagent positions, is created. This is evident by the default selected radio buttons.

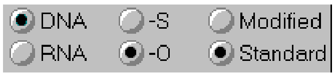
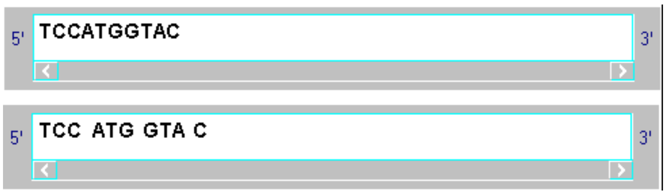


Figure 4-3. *Radio buttons in the Sequence page used for choosing the base type to be included in the sequence.*

By changing the radio button combination, you can freely change the component bases contained within the sequence. You are able to select DNA or RNA, whether the base is oxidated or thiolated, and whether the base is taken from the standard or modified reagent position. There are two extra physical reagent positions in OligoPilot II, labelled X and Y. The extra characters Z and Q are also provided. The available combinations are as follows:

Radio button combination	Bases as represented on the screen
DNA, **-O**(xidated), **Standard**	A, C, G, T, X, Y, Z, Q
DNA, **-O**(xidated), **Modified**	$\overline{A}$, $\overline{C}$, $\overline{G}$, $\overline{T}$
DNA, **-S** (thiolated), **Standard**	$\underline{A}$, $\underline{C}$, $\underline{G}$, $\underline{T}$, $\underline{X}$, $\underline{Y}$, $\underline{Z}$, $\underline{Q}$
DNA, **-S** (thiolated), **Modified**	$\underline{\overline{A}}$, $\underline{\overline{C}}$, $\underline{\overline{G}}$, $\underline{\overline{T}}$
RNA, **-O**(xidated), **Standard**	a, c, g, u, x, y, z, q
RNA, **-O**(xidated), **Modified**	$\overline{a}$, $\overline{c}$, $\overline{g}$, $\overline{u}$
RNA, **-S** (thiolated), **Standard**	$\underline{a}$, $\underline{c}$, $\underline{g}$, $\underline{u}$, $\underline{x}$, $\underline{y}$, $\underline{z}$, $\underline{q}$
RNA, **-S** (thiolated), **Modified**	$\underline{\overline{a}}$, $\underline{\overline{c}}$, $\underline{\overline{g}}$, $\underline{\overline{u}}$

5. Click on the **Group** button if you want the sequence to be displayed in groups of three bases, beginning from the 5´ end.



5' TCCATGGTAC 3'

5' TCC ATG GTA C 3'

Figure 4-4. *An ungrouped (top) and grouped (bottom) sequence in the sequence editor field of the Sequence page.*

6. To save the sequence you have created, click on the **Save As** button and type in a name for you sequence. The name can be up to 256 characters in length. Click on **OK**. The name of the saved sequence will now be displayed in the Sequence page containing the specific sequence. Note that saved sequences are personal to the current user, i.e. users logged in under a specific username will not see the saved sequences of another user.

7. Place a check mark in those boxes beside the **Optional method steps** that you want to be included in your method.

8. Create the method for the sequence you have entered by clicking on the **Create** button. The **Create** button serves four main purposes:
   - to check the sequence for invalid combinations (ignoring the 3´ base), e.g. it is not possible to include both base ‘A’ (DNA) and base ‘a’ (RNA) in the same sequence since they both take up the same reagent bottle position on the instrument.
   - to generate a method based on the sequence and cross-reference list (see Section 5.1.1).
   - update the method variables based on the generated method.
   - display the **Save As** dialogue so that the method can be saved before performing a run.

   Enter a name for the method, select the destination and click on **OK** (see Section 4.6 for more details). The method is saved with default values for the method variables. These can later be changed before you start a run (see Section 4.7 and Chapter 6) or you can change the variables and save the method under a new name.

Note: Users with the appropriate access authorization have global access to methods created by all users. In such circumstances, an already saved method can be used as the basis for generating a new method with a different sequence. This is particularly useful if, for example, a method was saved with specially modified blocks (see Section 5.2) or cross-reference lists (see Section 5.1.1).

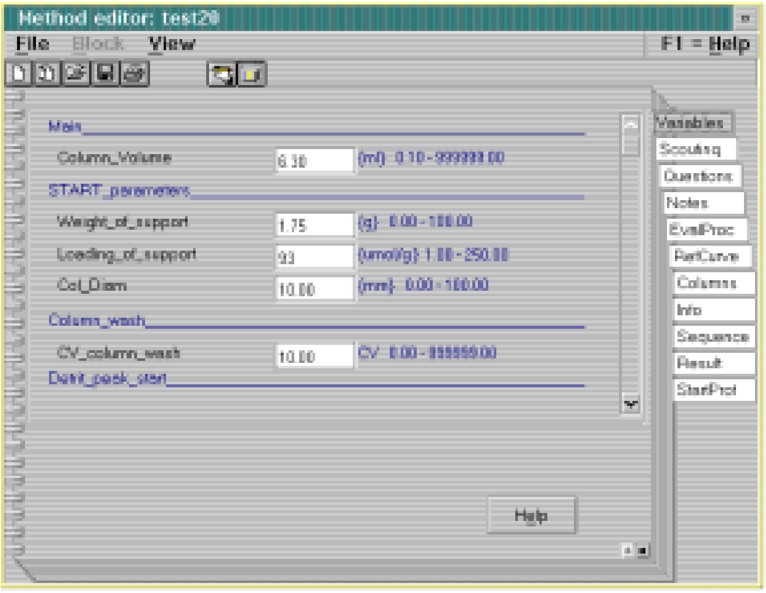
4.4 Editing method variables

Having created a method, you may want to change the default values for the method variables. The method templates contain all the blocks that are likely to be needed for a typical synthesis run. By defining a sequence and then creating a method, the program automatically builds up a method to synthesize the specific sequence by copying in all of the relevant blocks from the method template.

Each block has a set of method variables, displayed on the **Variables** page in Run setup. You set default values for the variables in the Method editor, and can change these values for a particular run when the run is started.

1. After you have created your method (see Section 4.3), select the Variables page in Run setup.

2. Work through the variable list, adjusting the values to suit your synthesis. To change a variable value, simply type the required value in the field. Remember that the values you enter here will be default values, suggested each time the method is run.



Main

Column_Volume 6.30 (ml) 0.10 - 999999.00

START_parameters

Weight_of_support 1.75 (g) 0.00 - 100.00

Loading_of_support 93 (umol/g) 1.00 - 250.00

Col_Diam 10.00 (mm) 0.00 - 100.00

Column_wash

CV_column_wash 10.00 CV 0.00 - 999999.00

Detrit_peak_start

Figure 4-5. *The Variables page in Run setup.*

If the whole variable list does not fit on one screen, a scroll bar (see Figure 4-5) will be shown to the right of the list. Click on the arrows at the ends of the bar to scroll one variable at a time, or on the bar itself to scroll one screen at a time. You can also drag the slider button to scroll, but this is not recommended since you can easily miss variables by scrolling too far.

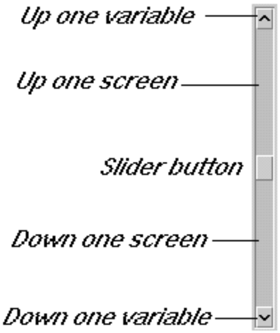


Figure 4-6. *Scroll bar functions.*

Typical variables are illustrated with the list below, taken from a method created for OligoPilot II with the **fixrec** method template. The list is organized according to the order of the blocks in the method. Other method templates may vary in their structure and variables.

- **Start_parameters**
  These variables together define the synthesis scale, i.e. Weight of the support, Loading of the support and Column diameter. CV (column volume) is also defined and is used for the calculation of special instructions such as Vol_Cap, Vol_amid and CT5_Cap.

- **CV_column_wash**
  The number of column volumes (CV) to wash the column is set here. If zero is entered, no wash will take place.

- **Detrit_peak_start**
  The flow rate of the detritylation solution is set here.

- **Detrit_wash**
  The pressure of the detritylation wash and the number of column volumes of detritylation solution to be used are set here.

- **DNA_Parameters**
  These variables together define the coupling of a base to the oligonucleotide sequence, i.e. how many equivalents of amidite should be added to the column with respect to the scale, the percentage volume of tetrazole to be used with respect to column volumes and the concentration of the amidite.

- **DNA_Recycle**
  The amidite recycling flow rate and time used are set here.

- **Oxidation_DNA**
  Oxidation stabilizes the phosphite group of the coupled amidite. The variables determine how many equivalents of iodine are used in the oxidation solution and the contact time between the oxidation solution and the support.

- **Capping**
  The unreacted 5´-hydroxyl groups on the oligonucleotide are capped to prevent further participation in the synthesis reaction. The column volumes of capping solution and the contact time are set here.

Note: The base for the all the blocks in the method templates, unless otherwise stated in the specific block, is column volume. To use absolute volume or time as the method base, change the **Base** instruction in the **Main** block using the text instruction editor. See Chapter 5 for a description of how to do this.

You can get a graphical view of the length of the blocks in a method as follows:

Text instructions

1. Click on the **Text instructions** button on the toolbar.

Windows

2. Click on the **Windows** button on the toolbar and select only **Graph**. The method is now displayed graphically.
3. Click on the **Scale** button in the graphical display to select a base for the graphical display. Changing the display base will not affect the base in the method.

Run setup

4. To return to the **Variables** page in Run setup, click on the **Run setup** button on the toolbar.

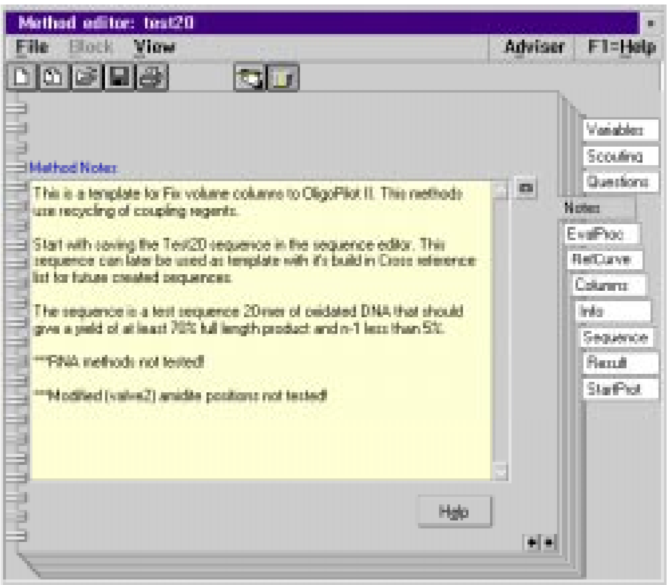
4.5 Method notes

Print

Maximize notes

Click on the **Notes** thumb-tab in the run set-up to show the **Notes** page, and read through the method notes. You can maximize each section in the notes page to fit more of the text on one screen. Click on the printer icon or choose **File:Print** to print the method notes.

The method notes provided with each template describe the important information about the template and, if relevant, how the system should be connected for the method to work correctly.



This is a template for Fix volume columns to OligoPilot II. This methods use recycling of coupling regents.

Start with saving the Test20 sequence in the sequence editor. This sequence can later be used as template with it's build in Cross reference list for future created sequences.

The sequence is a test sequence 20mer of oxidated DNA that should give a yield of at least 70% full length product and n-1 less than 5%.

**RNA methods not tested!

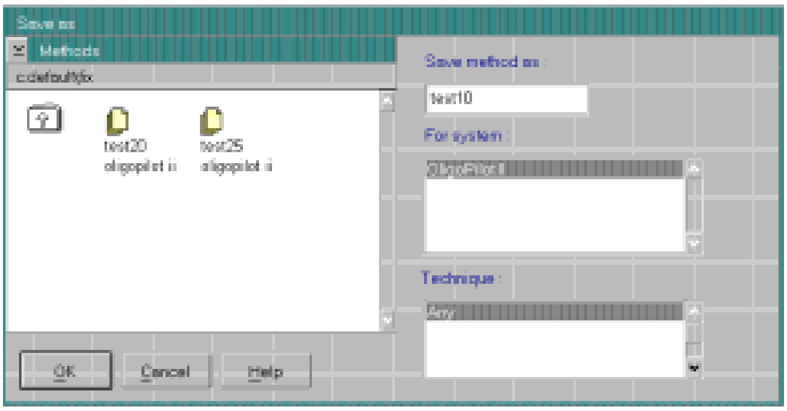
**Modified (valve2) amidite positions not tested!

Figure 4-7. The Notes page in run set-up with the method notes maximized.

If your system does not correspond to the description, either rearrange the valves and tubing connections in accordance with the method notes description or edit the method instructions (see Chapter 5) in accordance with your system set-up.

4.6 Saving the method

When you click on the **Create** button in the Sequence editor page, UNICORN OS checks the sequence for invalid combinations, generates the method, updates the method variables and automatically opens the **Save As** dialogue box. The method must be saved before it can be run.



Save method as : test10

For system : OligoPilot II

Technique : Any

Figure 4-8. *Dialogue box for saving a method. The Methods panel will be shown in icon or detail view according to the current setting in the Main menu. You can change the display mode from the pull-down menu, activated by clicking with the right mouse button in the panel (see Section 3.2.3).*

1. Enter a name for the method. Method names may be up to 8 characters long and may contain letters (A-Z), digits (0-9) and underscore characters. The case of letters is not significant. The method name must be unique for the chosen system within the directory (see steps 2 and 3 below).

2. By default, the method will be saved in your home directory. To change the directory, double-click on the appropriate directory icon in the **Methods** panel.

3. If you have more than one system connected to the PC, choose the system for which the method is intended. The method can only be run on the system for which it is saved. Remember that different systems may have different configurations and control capabilities.

4. Choose the technique for which the method was written.

5. Click on **OK**.

Note: The method templates are written for standard strategies. If you receive a syntax error message when the method is saved, one or more instructions in the method are invalid. These may be calls to blocks which are not defined, or instructions which are invalid in your customized strategy (this can also arise if a method is written for one system then saved on another). Invalid instructions are marked in red in text instruction mode in the Method editor (see Section 5.4.1), and must be deleted or replaced before the method can be run.

Save

The method remains open in the Method editor when it has been saved, so that you can continue editing if you wish. If you edit any of the method instructions or change the method variables, you can save these changes by selecting **File:Save** or clicking on the **Save** button in the toolbar. If you want to save a copy of the method under a new name, choose **File:Save As** and enter the details as described above. If you close an open method or the Method editor module and there are unsaved changes, you will be prompted to save these changes.

4.7 Starting a run

This section briefly summarizes how to start a run with a method. The method must be named and saved before it can be started. See Chapter 6 for more details of how to run a method.

Note: If you are editing the method in the Method editor and have made changes that you have not yet saved, these changes will not apply during the run. Similarly, if you edit the method while it is running, the run will not be affected. It is the version of the method which is saved on disk *at the time when the method is started* that controls the run. You are able change the method variables in the Start Protocol at the beginning of a run but these will only be valid for the current run and will not be saved.

1. Establish a control mode connection to the system where the method is to be run. See Section 6.5 for details. You cannot start a method without a control mode connection to the appropriate system.

2. Choose **File:Run** from the System control window for the required connection and select the method to run. Alternatively, select the method in the methods panel of the Main menu. (Do not double-click on the method icon in the Main menu: this will open the Method editor.)

3. Click on **Run**. This will display the first page of the start protocol.

4. Change the method variable values if required, such as loading weight, CV, column volume etc. The suggested values are those saved in the method. Any changes you make will apply only for the current run, and will be recorded in the run documentation.

5. Go through the rest of the start protocol, entering information where appropriate. Use the **Next** and **Previous** buttons to move through the start protocol. If you click on **Cancel** on any page in the start protocol, the method will remain loaded in the System control module but will not start. Start protocol pages for most method templates are:

   Method Variables All the variables defined in the method instructions, organized by block. Values for variables can be entered or changed here for the current run.

   Prerun Questions Questions are data entry fields which are filled in by the operator when the run is started. Some questions may be mandatory and some may require authorized confirmation.

   Notes Method notes are displayed and start notes can be entered.

   Method Information Information about the method being run.

   Oligo synthesis sequence The sequence of the oligonucleotide to be synthesized.

   Result name changeable The name of the result file is displayed here. This page is always displayed, and the name may be changed if this is permitted in the start protocol.

6. The last page of the start protocol has a **Start** button instead of **Next**. Click on this button to start the run.

4.8 Editing text instructions

Text instructions

Methods for most purposes can be created by adjusting the method variable values as described above. The method is, however, actually programmed as a series of instructions which use these variables as parameters. To see and/or change the instructions, click on the **Text instruction** button on the Method editor toolbar (or choose **View:Text instructions** from the menu). To see an overview of block the structure and buffer concentration in the method, choose **View:Windows:Block** and **View:Windows:Graph** respectively in the text instruction window (see Section 5.2).

With the instruction editor, you have complete facilities for designing and editing your own customized methods. You will also use the instruction editor for refining and modifying methods based on the standard templates, e.g.

••Changing the method base (column volume, volume or time).

••Changing method specifications (if the templates do not suit your system configuration).

••Adding or removing variables.

••Adding conditional events (such as starting peak integration when a certain peak level is detected).

••Adding or removing instructions to change the method functionality.

••Adding or removing blocks to change the method structure.

To gain an understanding of how method templates are built up and can be modified, work through Chapter 5, which gives a full description of method editing facilities.

5. Creating and editing methods

This chapter describes the complete facilities for creating and editing methods in UNICORN OS. Refer to Section 2.3 for an overview of method concepts. For many applications, suitable methods can be created by changing the default variable values in one of the templates supplied with UNICORN OS (see Section 4.4).

New methods can also be created using the more advanced editing facilities. These can be used for:

- changing selected instructions in the method templates (e.g. changing the method base)

- adding blocks and instructions (e.g. WATCH instructions)

- changing method instructions to adapt to non-standard system configurations

- creating new methods for applications which are not covered by the templates supplied

Advanced editing facilities can be used at three different levels, with the level chosen being dependent on the type and extent of method modifications to be made. These are:

- modifications at the sequence editor level, i.e. creating custom methods by assigning a specific base in the sequence editor to a freely chosen method block (see Section 5.1).

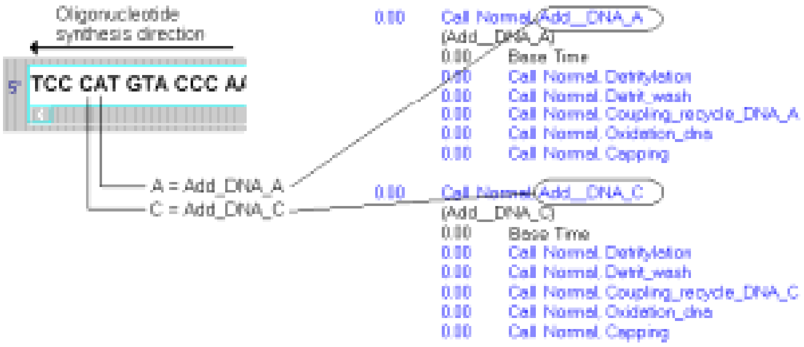
- adding new blocks to an existing method and/or modifying instructions in existing blocks (see Section 5.2)

- writing a new method “from scratch”, i.e. selection of **No Template** in the **New Method** dialogue box (see Section 5.9).

5.1 The sequence editor

The sequence editor in the Sequence page of Run setup is a user interface which allows methods to be easily created. The bases in a sequence are each cross referenced to a specific block in the method template, with each block representing a series of instructions to be performed. Thus, in entering a sequence into the sequence editor and clicking on the **Create** button, the specific blocks that are cross-referenced to the bases are copied from the method template into the method. The method must be saved before it can be then used to run an instrument.

For example, in a sequence containing the bases 5´-...CA...3´, the DNA base A is assigned to a specific block in the method template called **Add_DNA_A**. Consequently, by clicking on the **Create** button in the Sequence page, the A base in the sequence causes the block **Add_DNA_A** to be copied into the method. The next base in the sequence, DNA base C, is assigned to the block name **Add_DNA_C**. Thus, the block cross referenced to base C will next be added to the method (see Figure 5-1). In the standard method templates, each block that is cross-referenced to a base is a self-contained set of instructions to perform a complete coupling cycle of one base, i.e. detritylation, detrit wash, coupling, oxidation or thiolation and capping (see Figure 5-1).



```
0.00    Call Normal.Add__DNA_A
        (Add__DNA_A)
        0.00    Base Time
        0.00    Call Normal. Detritylation
        0.00    Call Normal. Detrit_wash
        0.00    Call Normal. Coupling_recycle_DNA_A
        0.00    Call Normal. Oxidation_dna
        0.00    Call Normal. Capping
0.00    Call Normal.Add__DNA_C
        (Add__DNA_C)
        0.00    Base Time
        0.00    Call Normal. Detritylation
        0.00    Call Normal. Detrit_wash
        0.00    Call Normal. Coupling_recycle_DNA_C
        0.00    Call Normal. Oxidation_dna
        0.00    Call Normal. Capping
```

Figure 5-1. *The relationship between the bases entered into the sequence editor and the blocks copied into the method. In the example, the sequence base A is cross referenced to the block name Add_DNA_A, which is then copied into the method. Similarly for the following base C, this is cross referenced to the block named Add_DNA_C which is also copied into the method. Note that each block contains a self-contained set of instructions for the complete coupling cycle of the specific base.*

5.1.1 Modifying cross references in the sequence editor

Cross referencing a base to a method template block lends much flexibility to modifying the method creation process. UNICORN OS allows the user to change the block that is cross-referenced to any specific base type.

For example, you may decide that the DNA sequence 5´-ACTGGT-3´ should have a column wash step after the addition of each base in the sequence. By cross referencing a base not used in the current sequence, e.g. A, to the column wash block in the method template, this can be directly incorporated into the sequence to signal a column wash. Thus, in the sequence 5´-AAACATAGAGAT-3´, the appearance of A does not mean that a thiolated DNA-A base will be added to the oligonucleotide, but rather a column wash procedure is performed after the addition of the preceding base.

Alternatively, you may modify the instructions in an existing block or create a new block. It is thus a straightforward task to assign the new block to the base in question.

To change the assignment, do the following:

New method

1. Create a new method by clicking on the **New Method** toolbar button or choose the **File:New:Method** menu command in the Method editor, or by choosing **File:New:Method** or **New:Method** in the Main menu. These alternatives are equivalent. When you choose the command from the Main menu, the Method editor is opened automatically. The **New Method** dialogue box will be displayed.

2. Choose the system for which the method is intended, e.g. OligoPilot or OligoProcess. From the **Technique** pull-down list, select **OligoPilot II** or **OligoProcess**. Next, select one of the available method templates in the **Template** pull-down list. Finally, choose **Any** in the **Column** pull-down list.

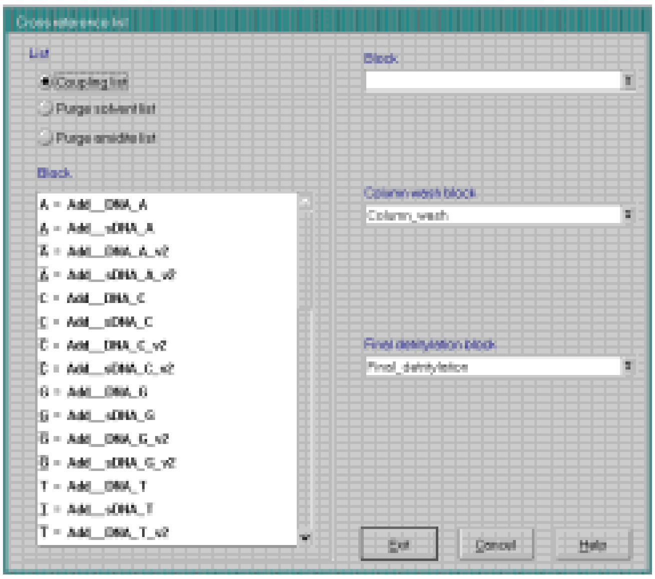
3. Click on **OK** once you have made your selections. The method template will now be opened as an untitled method.

Run setup

4. Click on the **Run setup** button or select **View:Run setup**. Select the Sequence page.

Cross references...

5. Click on the **Cross references** button to display the **Cross reference list** dialogue box.



List
● Coupling list
○ Purge solvent list
○ Purge amidite list

Block
A = Add__DNA_A
A = Add__sDNA_A
A = Add__DNA_A_v2
A = Add__sDNA_A_v2
C = Add__DNA_C
C = Add__sDNA_C
C = Add__DNA_C_v2
C = Add__sDNA_C_v2
G = Add__DNA_G
G = Add__sDNA_G
G = Add__DNA_G_v2
G = Add__sDNA_G_v2
T = Add__DNA_T
T = Add__sDNA_T
T = Add__DNA_T_v2

Block:

Column wash block: Column_wash

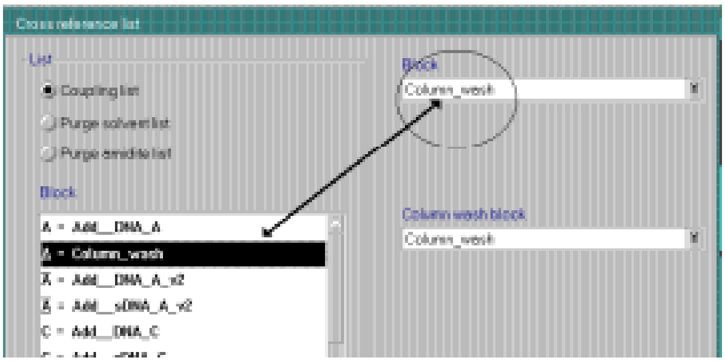
Final detritylation block: Final_detritylation

__Figure 5-2.__ Cross reference list dialogue box.

Select the **Coupling list** radio button in the **List** field. In the **Block** field you will see the all the bases and the block name that each is cross referenced to in the method template. For example:

```
A = Add_DNA_A
```

6. To change the assignment of a base to a different block in the method template, first select the base in the **Block** field. Next, click on the **Block** pull-down list and select the method template block that you want assigned to the selected base. The base in the **Block** field will now be associated with the block name that you chose.



List
● Coupling list
○ Purge solvent list
○ Purge amidite list

Block
A = Add__DNA_A
A = Column_wash
A = Add__DNA_A_v2
A = Add__sDNA_A_v2
C = Add__DNA_C

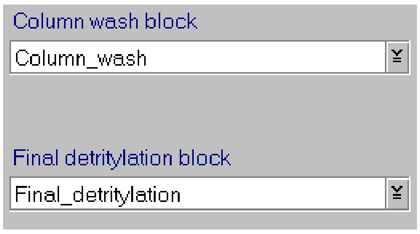
Block: Column_wash

Column wash block: Column_wash

__Figure 5-3.__ In the above example, the base A has been cross referenced to the block Column_wash.

You can select any block contained in the method template, even including those that you have modified or created (see Section 5.3). Repeat this process for other bases as appropriate.

7. You can also reassign the cross references for the optional method steps selected in the Sequence page. The blocks for column wash and final detritylation can be assigned using the pull down lists for **Column wash block** and **Final Detriylation** respectively.



Column wash block

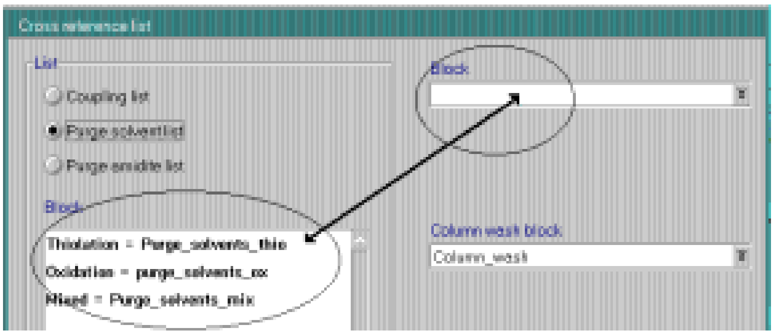
Column_wash

Final detritylation block

Final_detritylation

__Figure 5-4.__ Fields for cross referencing the column wash and final detritylation blocks in a method.

Additionally, you can click on the **Purge solvent list** and **Purge amidite list** radio buttons respectively to display the options in the **Block** field. These options can be cross referenced to different blocks in exactly the same way as described for bases in step 5.



Cross reference list

List

○ Coupling list
● Purge solvent list
○ Purge amidite list

Block

Thiolation = Purge_solvents_thio
Oxidation = purge_solvents_ox
Mixed = Purge_solvents_mix

Block

Column wash block

Column_wash

__Figure 5-5.__ Cross reference list of the purge solvent blocks available in a method.

7. Click on **Exit** to implement the new cross references.

8. Enter the appropriate sequence in the sequence editor field of the Sequence page. Create a method as described in Section 4.2.

9. Save the method (see Section 4.6).

Users with the appropriate access authorization have global access to methods created by all users. In such circumstances, an already saved method can be used as the basis for generating a new method with a different sequence. This is particularly useful if, for example, a method was previously saved with a specially modified cross-reference list. In such circumstances, open the appropriate method containing modified cross-reference list and access the Sequence page in Run setup. Delete the existing sequence in the sequence editor and enter the new sequence. Click on the **Create** button.

Note: Do *not* open a saved sequence from your personal sequence list in the Sequence page, otherwise the cross-reference list corresponding to the saved sequence will replace the modified cross-reference list present in the current method. Always manually enter your sequence over the original method sequence.

5.2 Text instructions editor

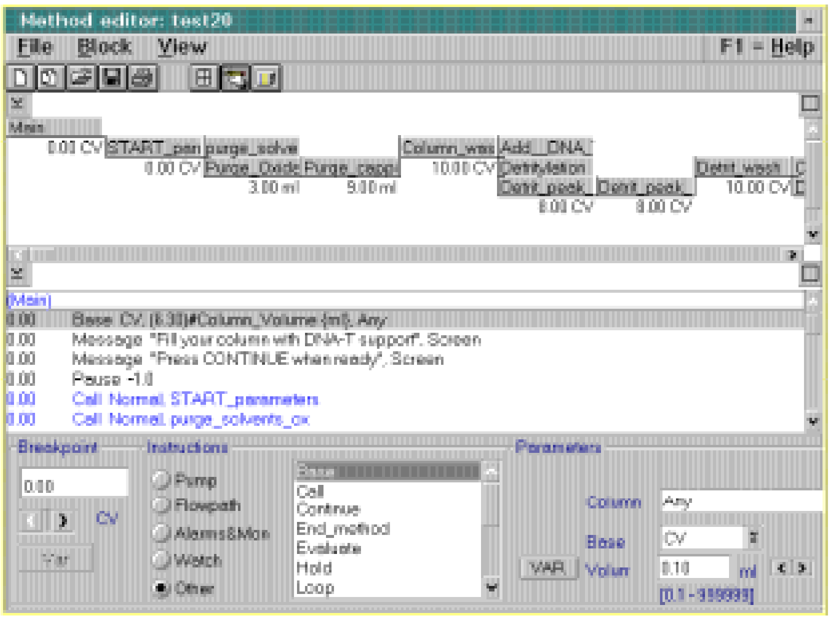
The next level of advanced editing to create new methods uses Text instructions. This involves modifying the instructions within the blocks of an existing method and/or adding new blocks to a method.

Note: Users with the appropriate access authorization have global access to methods created by all users. In such circumstances, an already saved method can be used as the basis for generating a new method with a different sequence. This is particularly useful if, for example, a method was previously saved with specially modified blocks.

This section introduces you to the Text instruction editor and the following sections present the components of a method and how to edit and create blocks.

Text instructions

The Text instruction editor is used for entering and editing method instructions. Click on the **Text** instruction**s** icon on the toolbar or choose **View:Text** Instruction**s** to display the Text instruction editor.



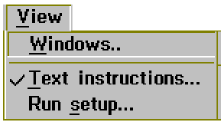
```
(Main)
0.00    Base CV, (6.30)#Column_Volume (ml), Any
0.00    Message "Fill your column with DNA-T support", Screen
0.00    Message "Press CONTINUE when ready", Screen
0.00    Pause -1.0
0.00    Call Normal, START_parameters
0.00    Call Normal, purge_solvents_ox
```

Breakpoint: 0.00 CV

Instructions: ● Other — Base

Column: Any
Base: CV
Volum: 0.10 ml [0.1 - 999999]

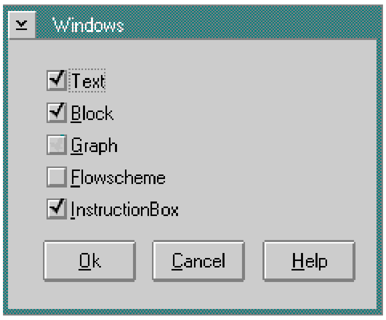
Figure 5-6. *The Method editor in Text instruction mode, showing the block window (top), Text instruction window (centre) and instruction dialogue box (bottom).*



⊞

View windows

Up to four windows can be displayed together with the instruction dialogue box. Click on the **Windows** toolbar button or choose the **View:Windows** menu command to open a dialogue box for choosing which windows to display.



☑ Text
☑ Block
☐ Graph
☐ Flowscheme
☑ InstructionBox

Figure 5-7. *The Windows dialogue box for selecting which windows to display in the Method editor.*

Method editing operations which can be performed in the various windows are summarized in the table below.

Window	Operations	See Section
Block window	Select block.	5.3
Text window	Display and hide block instructions. Select current instruction Move instructions within a breakpoint.	5.4
Graph window	Display block durations throughout the method. Not recommended for use with UNICORN OS.	-
Flow scheme window	For information only.	5.4.5
Instruction box	Specify breakpoints, instructions, parameters and variables. Insert, change and delete instructions.	5.4.2 - 5.4.4

5.3 Method blocks

Method blocks represent “modules” in the structure of the method. Most commonly, each block handles one functional unit within the method (e.g. column washing, amidite coupling etc.). Blocks are “called” either from MAIN (the top level of the method) or from other blocks using CALL instructions (unconditional) or WATCH instructions (conditional) (see Section 5.8.7). Calling a block executes the instructions in the block. A single method can contain up to 250 blocks.

Use text instruction mode to view and edit blocks.

5.3.1 Viewing blocks

In the block window
The organization of blocks in the method is shown graphically in the block window in Text instruction mode.

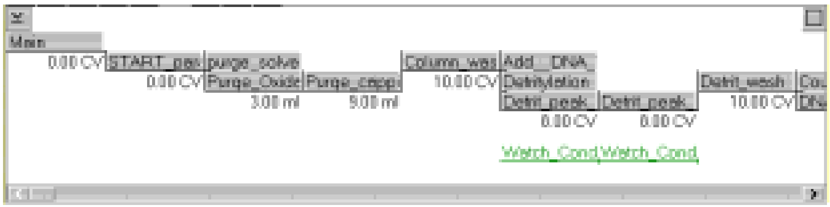


Figure 5-8. *The Method editor block window.*

Each block is represented by a grey line with the block name and the length of the block. The line is shifted down to indicate calls to other blocks. In the example above, the blocks are called in sequence from the Main block at breakpoint 0. Blocks to which there is no valid call are not shown in this window.

Conditional (WATCH) instructions are indicated by a green line showing the start and duration of the watch.

In the Text instruction window

In the text instruction window, each block is represented by a CALL or WATCH instruction in blue text. Double-click on the blue instruction to show the instructions in the called block (see Section 5.4.1). If you click on the line representing a block in the block window, the first instruction in the block will be highlighted in the text window.

5.3.2 Calling blocks

Blocks are activated in a method with the CALL instruction. A call to a block passes process control to the instructions in the block: when the block either finishes or executes an END_BLOCK instruction, control returns to the point from which the call was made.

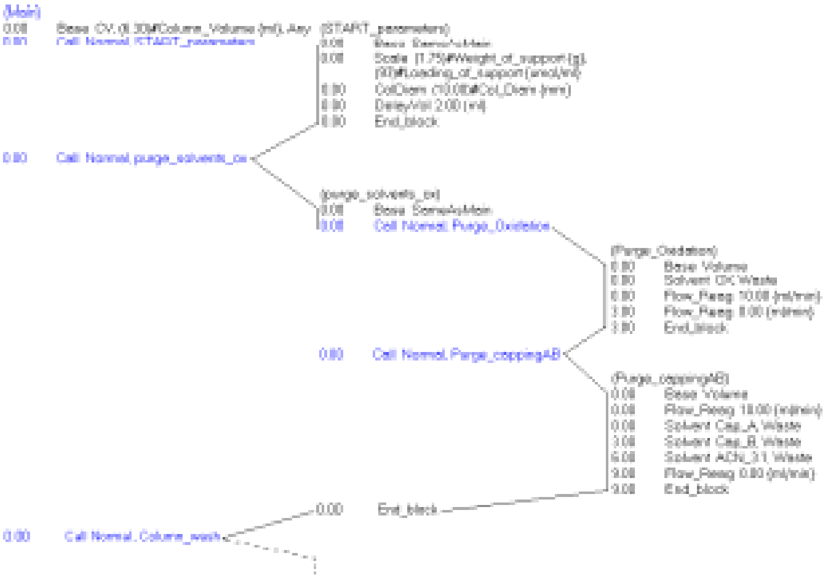

(Main)
0.00 Base CV (0.30)#Column_Volume (ml) Any
0.00 Call Normal START_parameters
(START_parameters)
0.00 Base SameAsMain
0.00 Scale (1.75)#Weight_of_support (g), (97)#Loading_of_support (umol/ml)
0.00 ColDiam (10.00)#Col_Diam (mm)
0.00 DelayVol 2.00 (ml)
0.00 End_block
0.00 Call Normal purge_solvents_ox
(purge_solvents_ox)
0.00 Base SameAsMain
0.00 Call Normal Purge_Oxidation
(Purge_Oxidation)
0.00 Base Volume
0.00 Solvent OX Waste
0.00 Flow_Reag 10.00 (ml/min)
3.00 Flow_Reag 0.00 (ml/min)
3.00 End_block
0.00 Call Normal Purge_cappingAB
(Purge_cappingAB)
0.00 Base Volume
0.00 Flow_Reag 18.00 (ml/min)
0.00 Solvent Cap_A Waste
3.00 Solvent Cap_B Waste
6.00 Solvent ACN_3.1 Waste
9.00 Flow_Reag 0.00 (ml/min)
9.00 End_block
0.00 End_block
0.00 Call Normal Column_wash

Figure 5-9. *Illustration of the flow of process control through method blocks.*

Calls may be of two types:

••*Unconditional calls* are made with the CALL instruction. Unconditional calls can be made in Normal or Restore mode, depending on how the system is to behave on return from the block. Normal mode retains any settings made in the block when the block is completed, so that the method continues using the settings which apply at the end of the block. Use of the Restore mode is not recommended.

••*Conditional calls* are made with a WATCH instruction, which sets a watch on a particular monitor signal and transfers control to a specified block if the signal meets a given condition. As long as the condition is not met, the block is not activated. There are different WATCH instructions for each process monitor signal, and each WATCH instruction can use various conditions to respond to absolute signal values or to rate of signal change.

- Note that the breakpoint when the WATCH instruction is issued determines when the watch begins, not when the block is activated. The block will in fact never be activated if the watch condition is not met during the run.

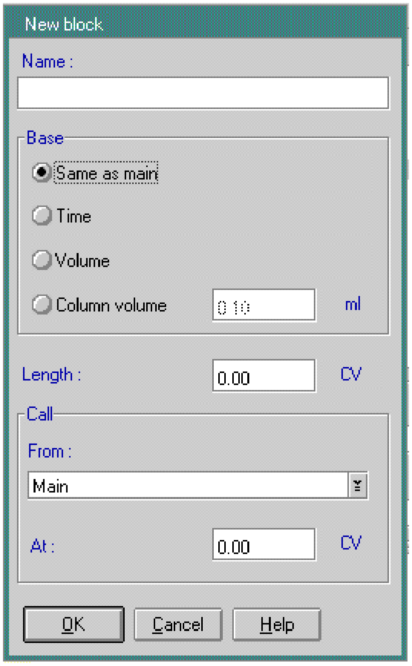
  Once set, a watch remains active until the condition is met or a new WATCH instruction is issued for the same monitor. The watch is cancelled automatically when the condition is met. A watch can also be turned off with the WATCH_OFF instruction.

  See Section 5.8.7 for more details of WATCH instructions.

5.3.3 Adding blocks

New block

To add a new block to a method, make sure the editor is in Text instruction mode, then click on the **New block** toolbar button or choose **File:New:Block** from the menu.



New block

Name :

Base

● Same as main

○ Time

○ Volume

○ Column volume 0.10 ml

Length : 0.00 CV

Call

From : Main

At : 0.00 CV

Figure 5-10. *The New block dialogue box.*

Block name

Enter a name for the block in the **Name** field. Block names may be up to 30 characters long, and may contain letters (A-Z), digits (0-9) and underscore characters. Use underscore characters instead of spaces if you want to separate words in a block name, e.g. Add_DNA_W. This field may not be left blank.

Block names must be unique within the method. The case of letters is retained but is not significant (the names Add_DNA_W and add_dna_w are treated as identical).

Base

Choose a base for the block. If you choose **SameAsMain**, the new block will inherit the base from the Main block in the method. The corresponding BASE instruction will be inserted in the block at breakpoint 0. If you choose **Column volume**, enter a value for the column volume.

In general, use **SameAsMain** wherever possible for the block base. You can then change the base of the whole method simply by changing the base of the Main block.

Length
You can enter a length for the block if required. An END_BLOCK instruction will be inserted in the block at the corresponding breakpoint. This field may not be left blank.

Call
You can create a CALL instruction to call the new block from an existing block (e.g. the Main block). Choose the block in which the CALL instruction is to be inserted in the **From** field and enter the breakpoint at which the CALL is to be made in the **At** field. If you do not want to create a CALL instruction (e.g. when the block being created is to be activated by a WATCH instruction), choose an empty line in the **From** field. Blocks which are created without a CALL instruction are placed last in the method, and the instructions are grey.

Notes: If the CALL instruction is placed at the same breakpoint as the END_BLOCK instruction, the CALL instruction will be placed immediately before END_BLOCK.

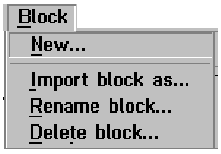
Do not call a block from within itself. You will generate a potentially infinite loop, which exceeds the maximum number of 10 calls allowed in a method. There is no limit for calls outside a loop.

Press **OK** to add the new block.

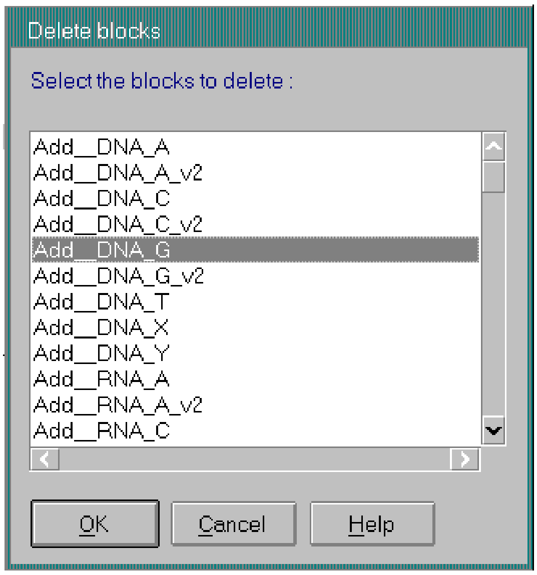
Strategy for creating blocks
For blocks which are to be called unconditionally, it is usually easiest to create the CALL instruction at the same time as you create the block, by selecting another block name in the **From** field in the **New block** dialogue box.

For blocks which are to be called conditionally with a WATCH instruction, do not create a CALL instruction at the same time as you create the block. Leave the **From** field empty in the **New block** dialogue box. When you later insert the WATCH instruction in the method, the new block name will appear in the list of possible actions for the watch. See Section 5.4.2 for details of inserting instructions.

5.3.4 Deleting blocks



To delete blocks from a method, choose **File:Delete Blocks**.


Delete blocks

Select the blocks to delete :

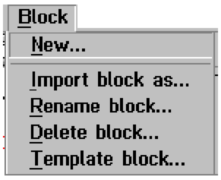
Add__DNA_A
Add__DNA_A_v2
Add__DNA_C
Add__DNA_C_v2
Add__DNA_G
Add__DNA_G_v2
Add__DNA_T
Add__DNA_X
Add__DNA_Y
Add__RNA_A
Add__RNA_A_v2
Add__RNA_C

Figure 5-11. The Delete blocks dialogue box.

Select one or more blocks to delete and press **OK**, then confirm or abandon the deletion. Double-clicking on a block name is equivalent to selecting the block and pressing **OK**.

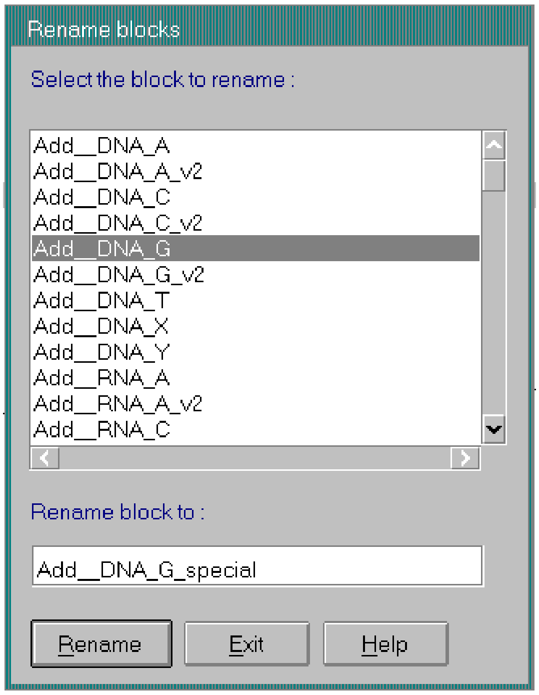
If you delete a block by mistake, you can restore the block by importing from the saved version of the method (see Section 5.3.6) provided that you have not saved the method with the block deleted.

Note: Any CALL or WATCH instructions which call deleted blocks remain in the method, but are shown in red in the Text instruction window to indicate that they are no longer valid. A method cannot be run if it contains invalid instructions.



5.3.5 Renaming blocks

To rename blocks, choose **File:Rename block**.



Add__DNA_A
Add__DNA_A_v2
Add__DNA_C
Add__DNA_C_v2
Add__DNA_G
Add__DNA_G_v2
Add__DNA_T
Add__DNA_X
Add__DNA_Y
Add__RNA_A
Add__RNA_A_v2
Add__RNA_C

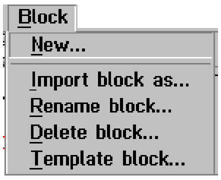
Rename block to : Add__DNA_G_special

Figure 5-12. *The Rename blocks dialogue box.*

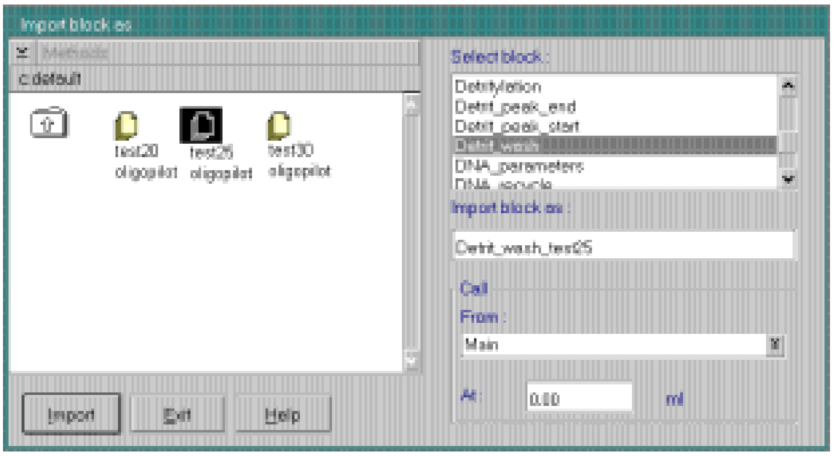
Select the block to rename, enter the new name in the **Rename Block** field and press **Rename**. The dialogue box remains open until you press **Exit**, so that you can rename more than one block without closing the dialogue box.

If the block you renamed is used in a CALL or WATCH instruction, the block name in these instructions will be changed automatically.

5.3.6 Importing and copying blocks



You can import blocks from other methods to the method you are developing. You can also use the import function to copy blocks within the method. Choose **File:Import block as**.



Import block as : Detrit_wash_test25

Call

From : Main

At : 0.00 ml

Figure 5-13. The Import block dialogue box.

Choose the method from which you wish to import and mark the block to import. The name of the selected block is suggested in the **Import block as** field. Click on **Import** to import the block. The dialogue box remains open until you click on **Exit**, so that you can import more than one block without closing the dialogue box.

Note: If you use the import function to copy blocks within a method, the blocks are copied from the saved version of the method on disk. Any changes you have made in the method but not yet saved will not be copied.

The imported block may not have the same name as an existing block in the method. If the default name is not allowed for this reason, the **Import** button will be grey and locked. Change the name of the imported block so that the **Import** button becomes available.

The block is imported exactly as it appears in the source method, and is placed after the last instruction in the text window, unless there is a call to the imported block already in the method. If any CALL or WATCH instructions in the imported block refer to non-existent blocks, the instructions will appear in red in the text window.

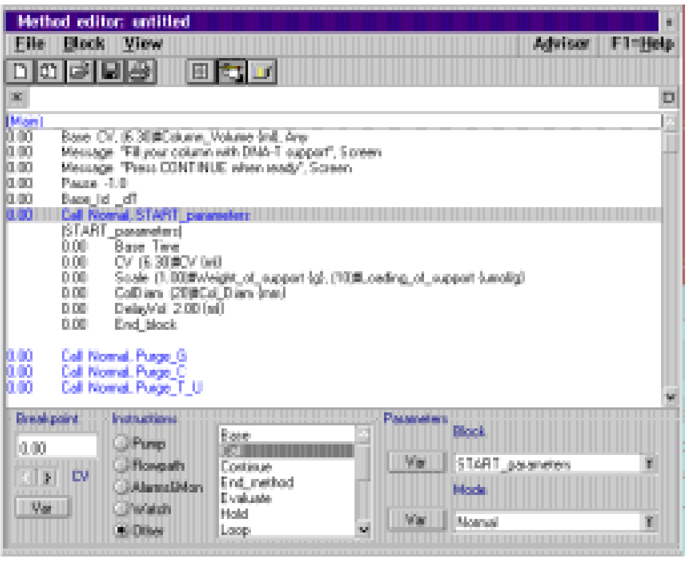
If the base of the imported block is defined as SameAsMain, the block will inherit the main base in the new method, regardless of the base in the source method.

5.4 Method instructions

Use the instruction dialogue box in Text instruction mode to enter, edit and delete instructions.

5.4.1 Viewing instructions

Instructions are displayed in the Text instruction window.



```
[Main]
0.00      Base CV, (6.30)#Column_Volume {ml}, Any
0.00      Message "Fill your column with DNA-T support", Screen
0.00      Message "Press CONTINUE when ready", Screen
0.00      Pause -1.0
0.00      Base_Id _dT
0.00      Call Normal, START_parameters
          [START_parameters]
          0.00      Base Time
          0.00      CV (6.30)#CV {ml}
          0.00      Scale (1.00)#Weight_of_support {g}, (10)#Loading_of_support {umol/g}
          0.00      ColDiam (20)#Col_Diam {mm}
          0.00      DelayVol 2.00 {ml}
          0.00      End_block

0.00      Call Normal, Purge_G
0.00      Call Normal, Purge_C
0.00      Call Normal, Purge_T_U
```

Figure 5-14. *The Text instruction window (top) with the instruction dialogue box (bottom).*

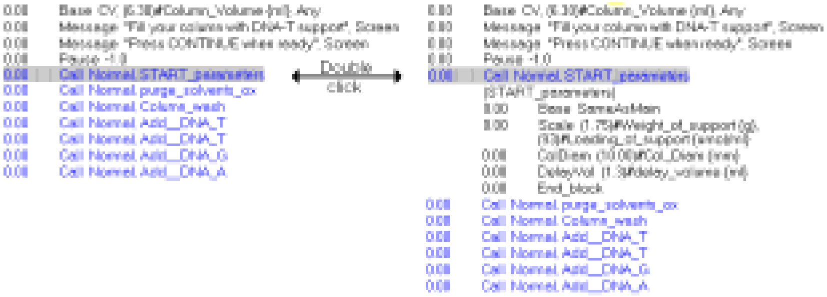
Instructions are colour-coded in the Text instruction window as follows:

Blue	Valid call instructions (i.e. CALL and WATCH instructions to other blocks in the method).
Black	Valid instructions other than CALL and WATCH.
Red	Instructions with invalid syntax. These may be: (a) calls to blocks which are not defined in the method, or (b) instructions which apply to a different system strategy (these can arise if a method is written for one system and saved for another). All red instructions must be deleted or changed before a method can be run (see Sections 5.4.3 and 5.4.4).

Grey	Instructions which will not be executed because they are either after the end of a block or method or constitute a block to which there is no call.

The current instruction is highlighted with a grey bar, and is also displayed in the instruction dialogue box where it can be edited (see Section 5.4.4). In working with instructions, you should display both the instruction dialogue box and the Text instruction window. Use the **View:Windows** command or toolbar button (see Section 5.2) to select windows to display. Alternatively, if the instruction dialogue box is not displayed, double-click on a black instruction in the Text instruction window to display the box.

Double-click on a CALL or WATCH instruction to display the instructions in the called block. If the instructions are already displayed, double-click on the call to hide the instructions in the called block. Double-clicking on the MAIN keyword at the beginning of the method will show or hide instructions in all blocks in the method.


```
0.00    Base CV, (6.30)#Column_Volume (ml), Any
0.00    Message "Fill your column with DNA-T support", Screen
0.00    Message "Press CONTINUE when ready", Screen
0.00    Pause -1.0
0.00    Call Normal START_parameters
0.00    Call Normal purge_solvents_ox
0.00    Call Normal Column_wash
0.00    Call Normal Add__DNA_T
0.00    Call Normal Add__DNA_T
0.00    Call Normal Add__DNA_G
0.00    Call Normal Add__DNA_A
```

```
0.00    Base CV, (6.30)#Column_Volume (ml), Any
0.00    Message "Fill your column with DNA-T support", Screen
0.00    Message "Press CONTINUE when ready", Screen
0.00    Pause -1.0
0.00    Call Normal START_parameters
        [START_parameters]
        0.00    Base SameAsMain
        0.00    Scale (1.75)#Weight_of_support (g),
                (93)#Loading_of_support (umol/g)
        0.00    ColDiam (10.00)#Col_Diam (mm)
        0.00    DelayVol (1.3)#delay_volume (ml)
        0.00    End_block
0.00    Call Normal purge_solvents_ox
0.00    Call Normal Column_wash
0.00    Call Normal Add__DNA_T
0.00    Call Normal Add__DNA_T
0.00    Call Normal Add__DNA_G
0.00    Call Normal Add__DNA_A
```

Figure 5-15. *Displaying and hiding block instructions.*

Instructions are indented for each call level (see illustration above).

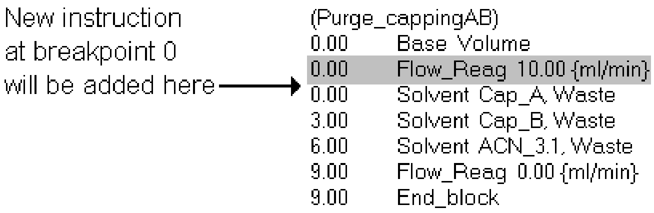
General oligosynthesis instructions are listed in Appendix B.

5.4.2 Adding instructions

To add a new instruction:

1. In the text instruction window, display the instructions in the block where you want to add a new instruction (see above).

2. Select an instruction in the block where you want to add the new instruction. If there are several instructions at the same breakpoint where you want to add the new instruction, select the instruction immediately before the position you want to add.



New instruction at breakpoint 0 will be added here

```
(Purge_cappingAB)
0.00    Base Volume
0.00    Flow_Reag 10.00 {ml/min}
0.00    Solvent Cap_A, Waste
3.00    Solvent Cap_B, Waste
6.00    Solvent ACN_3.1, Waste
9.00    Flow_Reag 0.00 {ml/min}
9.00    End_block
```

Figure 5-16. *Instructions added at the same breakpoint as existing instructions are inserted after the highlight.*

Note: Make sure that the current instruction in the text window is in the block, not the call to the block.

3. In the Instruction dialogue box:

   (a) Set the desired breakpoint in the **Breakpoint** panel.

   (b) Choose the instruction group and instruction in the Instruction**s** panel. Instructions are divided into 5 groups according to function: the list shows available instructions in the currently selected group. For short help on the purpose of each instruction, click on the instruction and press F1.

   (c) Enter values for instruction parameters in the **Parameters** panel. If a scroll bar appears on the right-hand side of the **Parameters** panel, additional parameters are required. The scroll bar blinks to alert you until you have accessed the additional parameters.

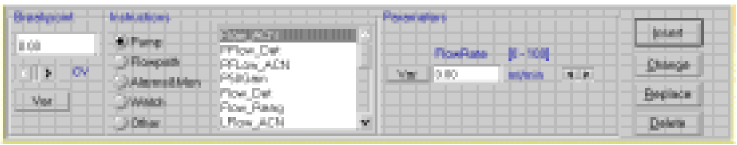


Figure 5-17. *The Method editor instruction box.*

4. Click on **Insert**. The new instruction will be inserted in the block either

   (a) at the position of the breakpoint of the new instruction if there are no other instructions at that breakpoint

   (b) immediately after the currently highlighted instruction if the highlight is at the same breakpoint as the new instruction

   (c) as the last instruction at the breakpoint if there are several instructions at the same breakpoint as the new instruction and none of these is highlighted.

Note: Instructions which are placed at the same breakpoint are executed simultaneously, with the exception of (i) CALL instructions, which are executed in the sequence in which they are written, and (ii) pump instructions, whereby the first pump instruction is followed and the rest ignored. If it is important that instructions at the same breakpoint are not executed simultaneously (e.g. when HOLD or PAUSE is used), place the instructions at breakpoints separated by 0.1 base units (see Section 5.8.2 for further details).

5.4.3 Deleting instructions

To remove an instruction:

1. Select the instruction in the Text instruction window.

2. Press **Delete** in the instruction dialogue box.

An instruction which has been deleted can only be recovered by re-inserting the instruction.

Note: You cannot delete the BASE instruction at the beginning of a block.

If you delete the END_BLOCK instruction, the block will end at the last instruction in the block.

5.4.4 Changing instructions

There are three possibilities for changing an instruction:

••change the breakpoint

••change parameters (including variables, see Section 5.5)

••select another instruction

To change an instruction:

1. Select the instruction in the Text instruction window. The instruction with its current parameters will appear in the Instruction box.

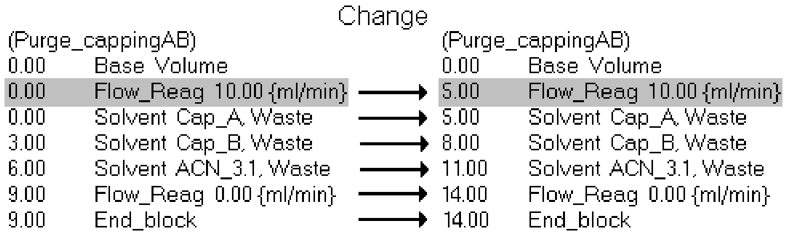
2. In the Instruction box, make the required changes to the breakpoint or parameters or choose a new instruction.

3. Press **Change** or **Replace**. These buttons are equivalent unless changes are made to the breakpoint or the length of a gradient

Changing breakpoints

Change and **Replace** have different functions if the breakpoint is changed:

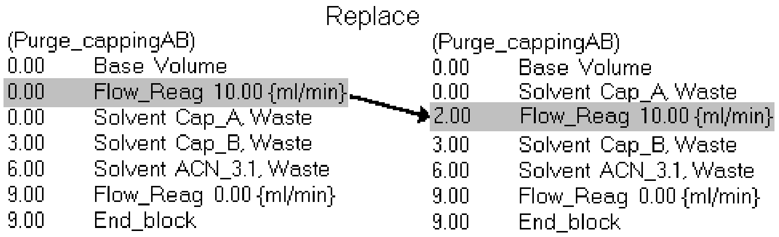
••**Change** shifts all subsequent instructions in the block according to the change in the breakpoint. **Change** does not affect the relative order of instructions in the method. You cannot change the breakpoint of an instruction to earlier than the nearest previous breakpoint in block.



```
                          Change
(Purge_cappingAB)                            (Purge_cappingAB)
0.00   Base Volume                           0.00   Base Volume
0.00   Flow_Reag 10.00 {ml/min}  ------>     5.00   Flow_Reag 10.00 {ml/min}
0.00   Solvent Cap_A, Waste      ------>     5.00   Solvent Cap_A, Waste
3.00   Solvent Cap_B, Waste      ------>     8.00   Solvent Cap_B, Waste
6.00   Solvent ACN_3.1, Waste    ------>     11.00  Solvent ACN_3.1, Waste
9.00   Flow_Reag 0.00 {ml/min}   ------>     14.00  Flow_Reag 0.00 {ml/min}
9.00   End_block                 ------>     14.00  End_block
```

***Figure 5-18.** Change moves the selected instruction and all subsequent instructions.*

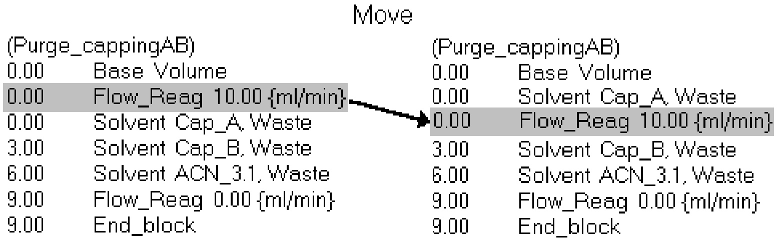
••**Replace** moves the selected instruction but does not change the breakpoint of any other instruction. **Replace** can change the relative order of instructions in the method.



```
                          Replace
(Purge_cappingAB)                        (Purge_cappingAB)
0.00   Base Volume                       0.00   Base Volume
0.00   Flow_Reag 10.00 {ml/min}          0.00   Solvent Cap_A, Waste
0.00   Solvent Cap_A, Waste              2.00   Flow_Reag 10.00 {ml/min}
3.00   Solvent Cap_B, Waste              3.00   Solvent Cap_B, Waste
6.00   Solvent ACN_3.1, Waste            6.00   Solvent ACN_3.1, Waste
9.00   Flow_Reag 0.00 {ml/min}           9.00   Flow_Reag 0.00 {ml/min}
9.00   End_block                         9.00   End_block
```

***Figure 5-19.** Replace moves only the selected instruction.*

Moving instructions within a breakpoint
To change the order of instructions within the same breakpoint in a block, mark the instruction to move with the *right* mouse button and drag the instruction to its new location, holding the right mouse button down. You can only move instructions in this way within a group of instructions at the same breakpoint.

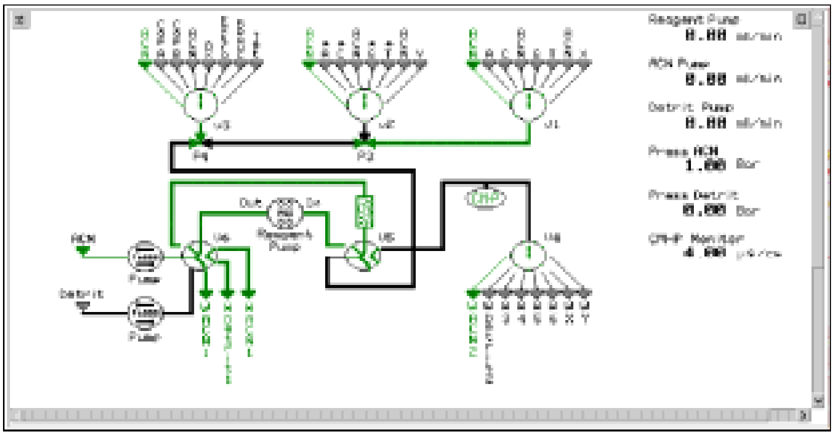

Move

```
(Purge_cappingAB)                              (Purge_cappingAB)
0.00    Base Volume                            0.00    Base Volume
0.00    Flow_Reag 10.00 {ml/min}               0.00    Solvent Cap_A, Waste
0.00    Solvent Cap_A, Waste                   0.00    Flow_Reag 10.00 {ml/min}
3.00    Solvent Cap_B, Waste                   3.00    Solvent Cap_B, Waste
6.00    Solvent ACN_3.1, Waste                 6.00    Solvent ACN_3.1, Waste
9.00    Flow_Reag 0.00 {ml/min}                9.00    Flow_Reag 0.00 {ml/min}
9.00    End_block                              9.00    End_block
```

Figure 5-20. *Instructions can be moved within the same breakpoint by dragging with the right mouse button.*

5.4.5 The flow scheme window

The flow scheme window displays the configuration of system components. This window is for information only, and can be useful e.g. in identifying valves for flow path instructions.



Reagent Pump
0.00 ml/min
ACN Pump
0.00 ml/min
Detrit Pump
0.00 ml/min
Press ACN
1.00 Bar
Press Detrit
0.00 Bar
CPHP Monitor
4.00 µS/cm

Figure 5-21. *The Method editor flow scheme window.*

5.5 Method variables

Variables can be assigned to any instruction parameter including breakpoints. Variable values can be changed immediately before the start of a run without using the Method editor, allowing one method to be used for runs under a variety of conditions (see Chapter 4).

Variables are defined with names which can be explicit descriptions of the variable function, e.g. Column_volume, Load_of_support. Suitable choice of variable names can make the method easier to read and understand, and also help the operator in setting variable values at the start of a run.

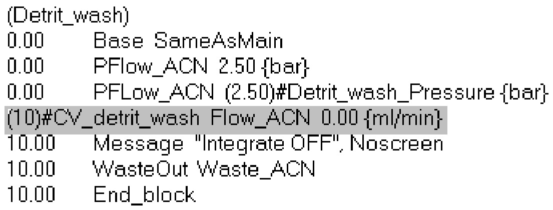
Each parameter defined as a variable is also assigned a default value, which is used if no changes are made to variable values at the start of a run.

Up to 64 variables can be included in any one block although only a total of 64 variables can be used in the whole method.

5.5.1 Identifying variables

Parameters defined as variables are identified in two ways:

••in the Text instruction window, the default value for the parameter is expressed by:
(variable value)#Variable_type{variable units} **.**



```
(Detrit_wash)
0.00     Base  SameAsMain
0.00     PFlow_ACN  2.50 {bar}
0.00     PFLow_ACN  (2.50)#Detrit_wash_Pressure {bar}
(10)#CV_detrit_wash  Flow_ACN  0.00 {ml/min}
10.00    Message  "Integrate OFF", Noscreen
10.00    WasteOut Waste_ACN
10.00    End_block
```

Figure 5-22. *Default values for variables appear in parentheses in Text instructions.*

••when the instruction is shown in the Instructions dialogue box, the **Var** button beside the parameter field is labelled with upper-case letters (**VAR**).

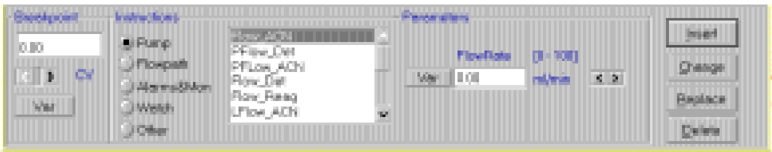


Figure 5-23. *Parameters with variable definition are identified by upper-case letters on the VAR button. In this example, the breakpoint is defined as a variable and the valve position is fixed.*

All variables are also listed on the **Variables** page of the Run setup (see Section 5.6.1), grouped according to the block in which they appear.

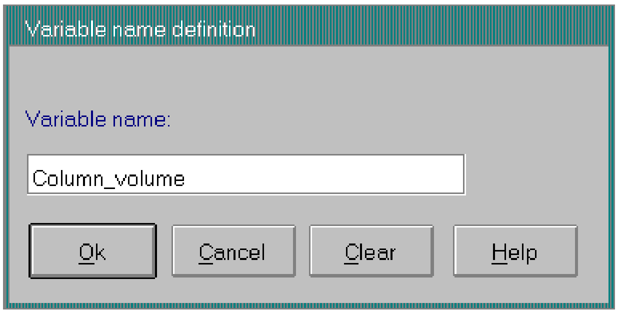
5.5.2 Defining variables

To define a new variable (i.e. convert an existing fixed value to a variable):

1. In the Text instruction window, select the instruction where you want to define the variable. The parameters for the instruction are shown in the instruction dialogue box.

2. Locate the breakpoint or the required parameter in the instruction box. Click on the **Var** button.

3. Enter a name for the variable in the dialogue box and click on **OK**.



Variable name: Column_volume

Figure 5-24. *The Variable name definition dialogue box.*

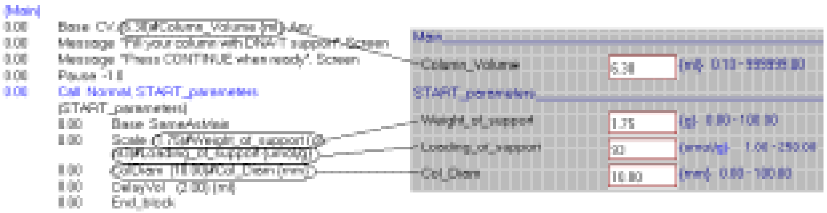
Variable names may be up to 32 characters long, and may contain letters (A-Z), digits (0-9) and the underscore character. Use underscore characters instead of spaces if you want to separate words in a name (e.g. Flow_rate). Names must be unique within the method.

The case of letters is retained but is not significant. The names Flow_Rate and FLOW_RATE are treated as identical.

When you define a variable, the value in the parameter field applies as the default value for the variable.

Note: Only one variable which affects block length may be defined within each block. Any number of other parameters may however be defined as variables within a block.

Default variable values can be changed either by editing the instruction in the instruction box or by changing the value in the **Variables** page of Run setup. Changes made in the Text instruction are automatically made in the **Variables** page and *vice versa* (Figure 5.25).



```
[Main]
0.00    Base CV (5.30)#Column_Volume {ml} Any
0.00    Message "Fill your column with DNA/T support" Screen
0.00    Message "Press CONTINUE when ready" Screen
0.00    Pause -1.0
0.00    Call Normal START_parameters
        [START_parameters]
        0.00    Base SameAsMain
        0.00    Scale (1.75)#Weight_of_support {g}
                (20)#Loading_of_support {umol/g}
        0.00    ColDiam (10.00)#Col_Diam {mm}
        0.00    DelayVol (2.00) {ml}
        0.00    End_block
```

Main

Column_Volume 5.30 {ml} 0.10 - 999999.00

START_parameters

Weight_of_support 1.75 {g} 0.00 - 100.00

Loading_of_support 20 {umol/g} 1.00 - 250.00

Col_Diam 10.00 {mm} 0.00 - 100.00

__Figure 5-25.__ Relationship between variables in Text instructions and in the Variables page of Run setup.

Important

If a breakpoint is defined as a variable, changing the variable value when the method is started will shift other instruction breakpoints accordingly. This functionality is equivalent to using Change to alter a breakpoint (see Figure 5-18 for an illustration of how Change affects instructions).

5.5.3 Removing a variable

To convert a variable to a fixed value:

1. In the Text instruction window, select the instruction where you want to remove the variable. The parameters for the instruction will be shown in the Instruction dialogue box.

2. Locate the required parameter in the instruction box. Press the **VAR** button.

3. Click on **Clear** to delete the variable name and click on **OK**.

5.5.4 Renaming a variable

To change the name of an existing variable:

1. In the Text instruction window, select the instruction where you want to rename the variable. The parameters for the instruction will be shown in the Instruction box.

2. Locate the required parameter in the instruction box. Press the **VAR** button.

3. Enter a new variable name in the dialogue box and click on **OK**.

5.6 Run setup

Run setup

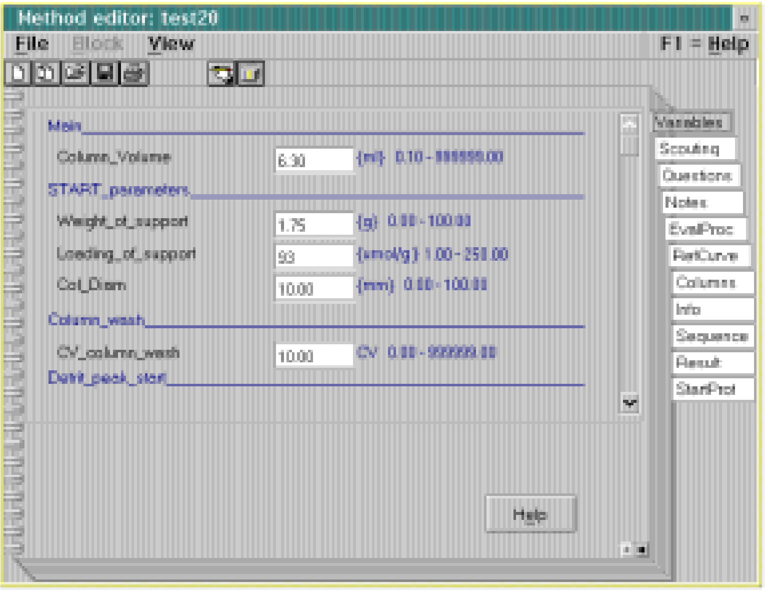
The Run setup is a series of pages for setting the behaviour of the method when it is started. To access the Run setup pages, press the **Run setup** button at the top of the Method editor toolbar.

To access a given page in the Run setup, click on the respective tab at the right of the Run setup screen. Alternatively press the small arrow buttons at the bottom right of each page to scroll forwards and backwards through the pages.

Note: Some of the pages in Run setup are only relevant for UNICORN version 2.10 used for chromatographic separations. These pages are not included in the descriptions.

5.6.1 Variables

The **Variables** page lists all variables used in the method with their default values, organized in order of the method blocks. You can change the default values to create a variant of the method (see Chapter 4).



Main

Column_Volume 6.30 {ml} 0.10 - 999999.00

START_parameters

Weight_of_support 1.75 {g} 0.00 - 100.00

Loading_of_support 93 {umol/g} 1.00 - 250.00

Col_Diam 10.00 {mm} 0.00 - 100.00

Column_wash

CV_column_wash 10.00 CV 0.00 - 999999.00

Detrit_peak_start

Figure 5-26. The Variables page in Run setup.

To change the default values, simply enter new values in the appropriate fields. Use the scroll bar to display additional variables if the variables occupy more than one screen. Click in the scroll bar to move one screen at a time, or on the arrows to move one variable at a time.

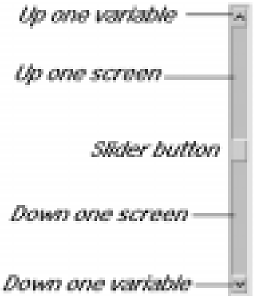


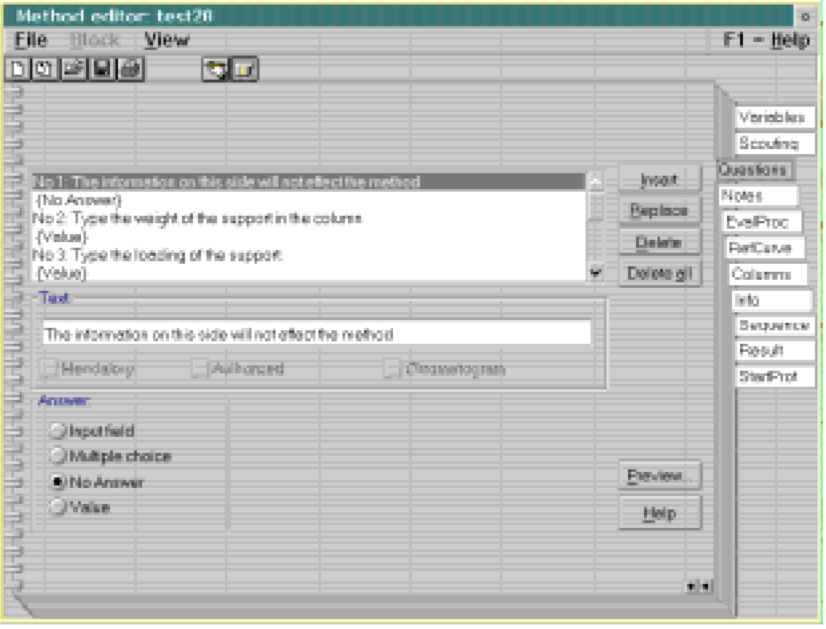
Figure 5-27. Scroll bar functions.

The changed values will be displayed for the corresponding instructions in the Text instruction window. Remember to save the method with the changed variables.

Note: The **Variables** box must be checked in the start protocol if you want to be able to change variable values at the start of a method (see Section 5.6.8).

5.6.2 Questions

Questions provides a means for entering structured run-specific information at the start of a run. Method templates supplied with UNICORN OS are defined with a set of questions for the synthesis scale, i.e. column volume, support load, weight of the support and delay volume. To define questions which will be shown when the method is started, open the **Questions** page in Run setup.



No 1: The information on this side will not affect the method
{No Answer}
No 2: Type the weight of the support in the column
{Value}
No 3: Type the loading of the support
{Value}

Text: The information on this side will not affect the method

□ Mandatory □ Authorized □ Chromatogram

Answer:
○ Input field
○ Multiple choice
● No Answer
○ Value

Figure 5-28. The Questions page in Run setup.

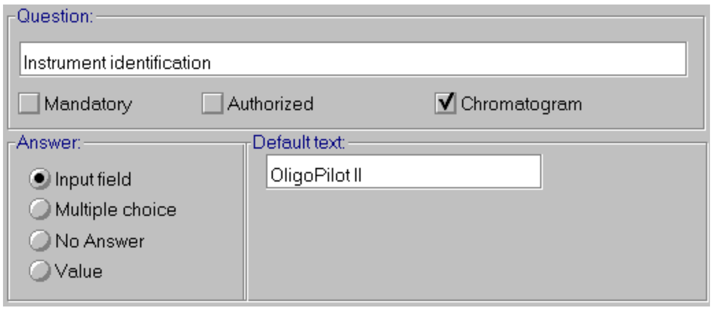
Questions may have the following status:

- **Mandatory**: these questions must be answered before a method is started.
- **Authorized**: answers to these questions must be acknowledged by a user with **Confirm/Unlock** authorization (see Section 12.2). The user's password must be given to acknowledge the answers.
- **Chromatogram**: these questions will be printed with the answers on the same page as the chromatogram if **Diagram Header** is chosen in an evaluation report.

Note: These options are non-exclusive, i.e. a question can be mandatory, require authorization and/or be printed on the chromatogram header.

Questions may be defined to accept four types of answers:

- **Input field** accepts any alphanumerical input as the answer. Input field questions may have a default answer. Example:



Question: Instrument identification

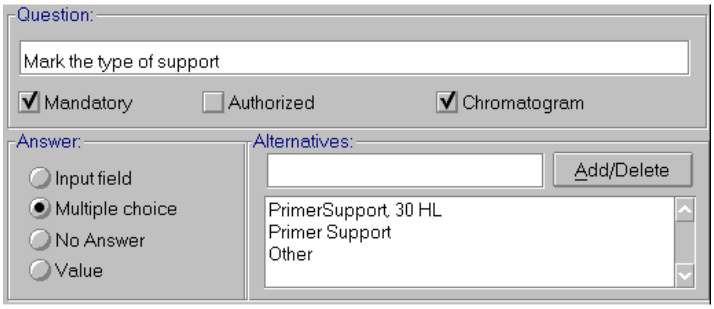
□ Mandatory □ Authorized ☑ Chromatogram

Answer:
● Input field
○ Multiple choice
○ No Answer
○ Value

Default text: OligoPilot II

__Figure 5-29.__ Options for input field questions.

- **Multiple choice** allows the user to choose one of a defined set of answers. To allow a blank answer, enter a space in one of the predefined answers. Example:



Question: Mark the type of support

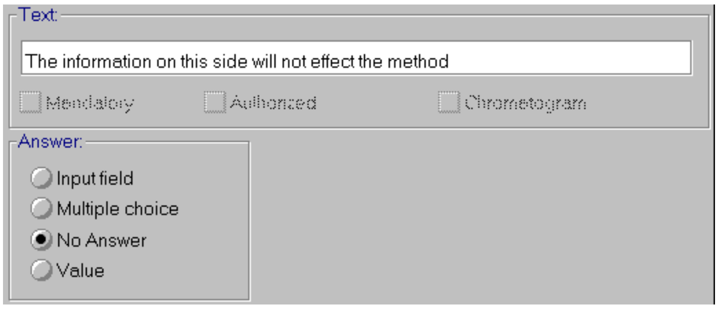
☑ Mandatory □ Authorized ☑ Chromatogram

Answer:
○ Input field
● Multiple choice
○ No Answer
○ Value

Alternatives:
PrimerSupport, 30 HL
Primer Support
Other

__Figure 5-30.__ Options for multiple choice questions.

- **No answer** does not require an answer. This kind of “question” may be to display important information or to split a question over more than one line (by setting all but the last line to **No answer**). Example:



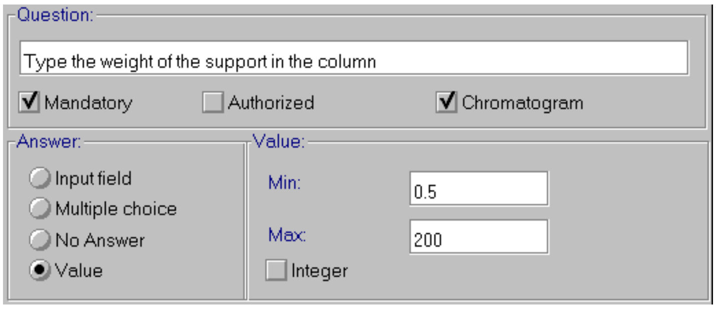
Text: The information on this side will not effect the method

□ Mandatory □ Authorized □ Chromatogram

Answer:
○ Input field
○ Multiple choice
● No Answer
○ Value

__Figure 5-31.__ Options for no answer questions.

••**Value** accepts only numerical answers. Value questions may have specified maximum and minimum limits, and may be defined to accept only integer values.



Question:

Type the weight of the support in the column

☑ Mandatory □ Authorized ☑ Chromatogram

Answer:

○ Input field
○ Multiple choice
○ No Answer
● Value

Value:

Min: 0.5

Max: 200

□ Integer

Figure 5-32. *Options for value questions*

Press the **Preview** button to display the questions as they will appear when the method is run. (Alternative answers to multiple choice questions are not shown in this mode). From preview mode, press **Edit** to return to question editing mode.

Inserting a question

To insert a new question after an existing question:

1. Click on the existing question in the questions list.

2. Enter the text, status, type and answer for the new question as required. The **Answer** panel depends on the type of question:

   ••Input field questions: Enter a default answer if required.

   ••Multiple choice questions: Click on the input field under **Alternatives**, enter the answer and click on **Add/Delete**. Repeat this procedure to add other alternatives. New alternatives are always added at the end of the list. To remove an alternative, mark the alternative in the scroll list and click on **Add/Delete**.

   ••Value questions: Enter maximum and minimum limits. Check the **Integer** box to if the question is to accept only whole numbers as answers.

3. Click on **Insert** to add the new question to the list.

If the list is empty, the **Insert** operation creates the first question in the list.

Editing an existing question

To change the definition of an existing question, select the question to be changed. Change the text, status, type and answer as required and click on **Replace**.

Deleting a question

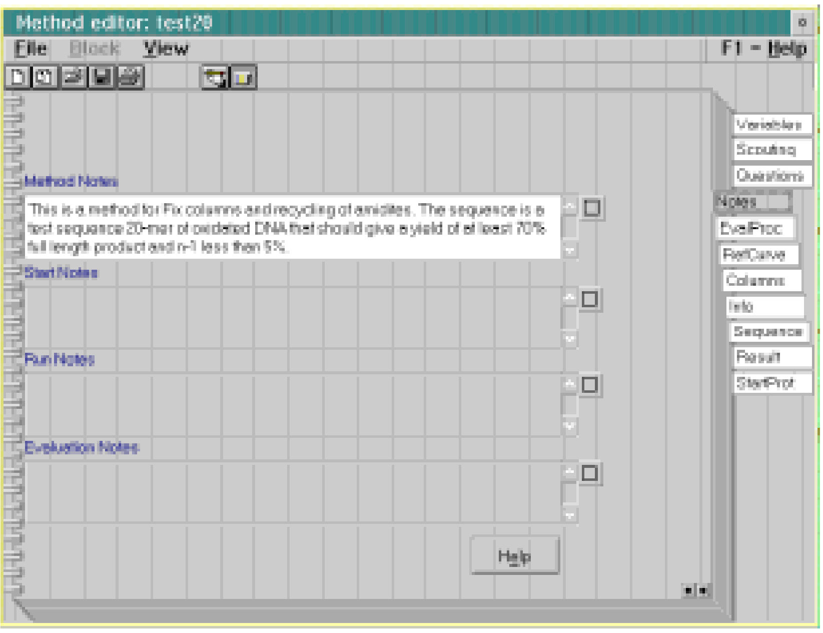
To remove a question, select the question and click on **Delete**. To remove all questions, click on **Delete All.**

5.6.3 Method notes

Notes are descriptive comments that form part of the method documentation. There are four separate notes fields for method editing, start-up, run and evaluation respectively. Only the method notes can be edited from the Method editor: the other notes are accessible at the respective stages in a run.

□ **Maximize notes**

To view the method notes, open the **Notes** page in the Run setup. Method templates are supplied with notes describing the system requirements for running the method. Read through these notes carefully before using a method. Click on the **Maximize notes** button to expand a notes field to fill the notes page. Click on the same button again to restore the default display with all four notes fields visible.



This is a method for Fix columns and recycling of amidites. The sequence is a test sequence 20-mer of oxidated DNA that should give a yield of at least 70% full length product and n-1 less than 5%.

Figure 5-33. *The Notes page in Run setup.*

The notes are entered as free text and may be edited using standard OS/2 editing functions to edit the notes. Words wrap automatically at the end of the field.

<Insert>	Toggles between insert and type-over mode. In type-over mode the cursor marks the current character.
<Shift+Del>	Cuts the marked text, saving it on the clipboard.
<Ctrl+Insert>	Copies the marked text to the clipboard.
<Shift+Insert>	Pastes the contents of the clipboard at the cursor.
<Delete>	Clears the marked text without saving it in the clipboard, or deletes the character to the right of the cursor if no text is marked.
<Backspace>	Clears the marked text without saving it in the clipboard, or deletes the character to the left of the cursor if no text is marked.

In the default method templates supplied with UNICORN OS, the method notes describe the system setup required by the method. We recommend that you use method notes for this purpose in your own methods, to provide documentation of the method requirements. Bear in mind that method notes are saved with the method and apply to all runs made with the method. Use the start or run notes for run-specific information.

The date and time when the method was created and last edited are saved automatically in the method information, and need not be entered in the method notes.

5.6.4 Evaluation procedures

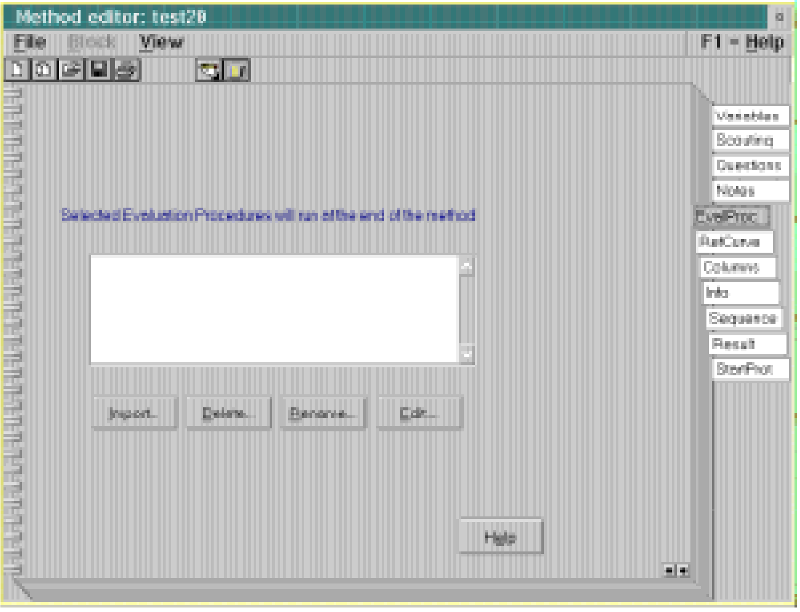
Evaluation procedures can be called automatically at the end of a method to evaluate and/or print the results.

User-defined procedures are created in the Evaluation module and may be saved in method files (see Section 7.6). Procedures saved with one method file can be imported to another.

Note: A procedure in a method will not be updated when a procedure with the same name is changed in Evaluation. The same applies to report formats saved in a procedure.

Note: If you use an evaluation procedure to print results automatically from a run controlled from a remote station in a network installation, the results will be printed on the printer currently set up on the local station, not on the remote station. If however you execute the procedure interactively from the

Evaluation module on the remote station, the results will be printed on the printer set up on the remote station where you are working.



Selected Evaluation Procedures will run at the end of the method

Figure 5-34. *The EvalProc page in Run setup.*

Defining and viewing procedures

Evaluation procedures are normally defined in the **Evaluation** module. Procedures imported to a method can also be viewed and edited in the Method editor: select the required procedure in the list and click on **Edit**.

Note: Evaluation procedures which process chromatogram data rely on consistent identification of curves in the result file for correct operation. If you include evaluation procedures with a method, make sure that references to curves in the procedure will be valid when the procedure is executed at the end of the run.

Selecting procedures to run

The **EvalProc** page lists all evaluation procedures associated with the method. Click on the procedure(s) which are to be executed at the end of the run. The procedures will be executed in the order they appear in the list.

Importing procedures
To import an evaluation procedure:

1. Select the **EvalProc** page and click on **Import**.

2. Choose a procedure from the **Select** list. You can also choose to import a procedure from another method. Select a method in the **Methods** panel to show the procedures stored in the method. If you have chosen a method, click on **Eval Procedures** to return to the complete list.

3. If desired, change the procedure name in the **Import as** field.

4. Click on **Import**.
   The dialogue box remains open until you click on **Exit**, so that you can continue to add procedures from the same or different method files.

Deleting procedures
To remove one or more procedures from the method:

1. Select the **EvalProc** page and click on **Delete**.

2. Select one or more procedures from the list and click on **OK**.

Note: Procedures that you delete from the method are removed from the method file when you save the method.

Renaming procedures
To rename a procedure in a method:

1. Select the **EvalProc** page and click on **Rename**.

2. Select a procedure from the list and change the name in the **Rename item to** field.

3. Click on **Rename**.
   The dialogue box remains open until you click on **Exit**, so that you can rename more than one procedure without closing the dialogue box.

Editing procedures
To edit a procedure in a method:

1. Select a procedure on the **EvalProc** page and click on **Edit**.

2. Edit the procedure.

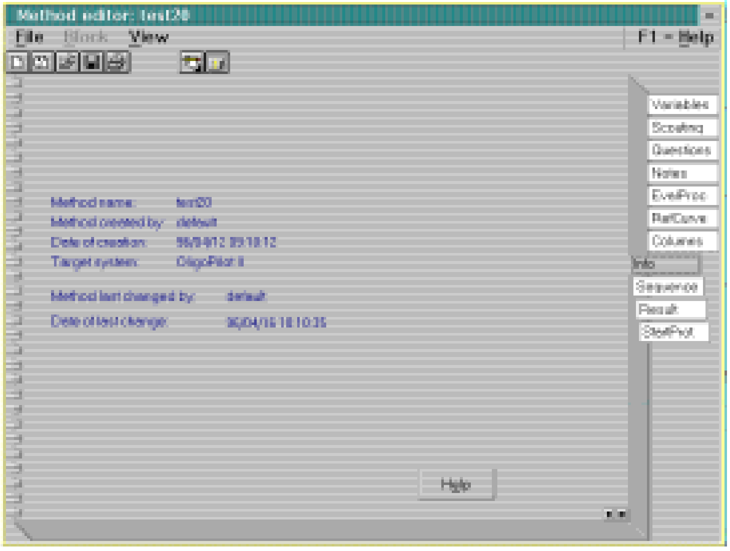
3. Choose **File:Exit** from the procedure editor menu. (**File:Save** is not available in the procedure editor window when you edit procedures in a method. Changes are saved automatically when you close the procedure editor).

Note: Report formats in procedures cannot be edited or viewed.

5.6.5 Info

The **Info** page displays information about the method, including method name, username, date for creation, target system and most recent modification.

The **Info** page is for information only and cannot be edited.



Method name: test20
Method created by: default
Date of creation: 96/04/12 09:18:12
Target system: OligoPilot II

Method last changed by: default
Date of last change: 96/04/16 18:10:35

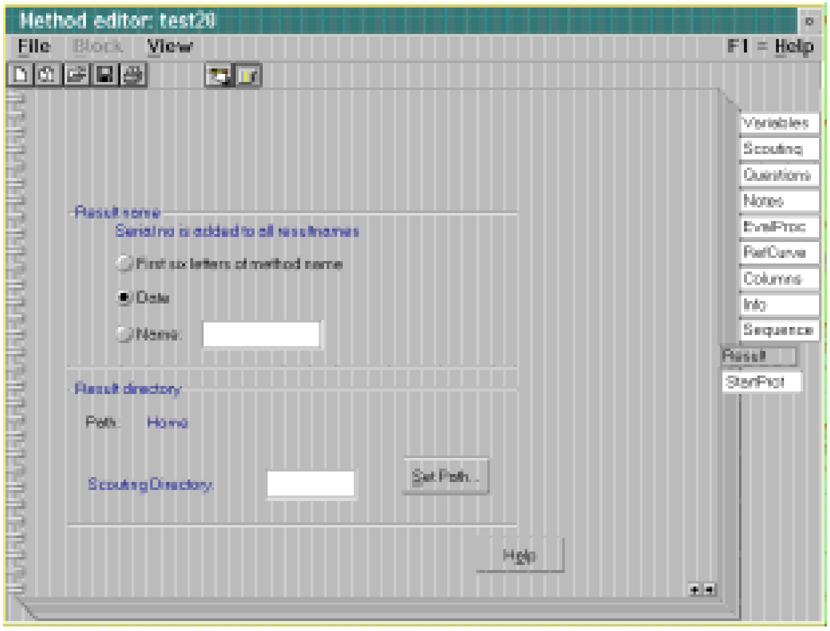
__Figure 5-35.__ The Info page in Run setup.

5.6.6 Sequence

The Sequence page contains the user interface between the desired sequence to be synthesized and the method created to run the synthesis procedure. The properties of this interface are described in Chapter 4 and Section 5.1.

5.6.7 Result

Use the **Result** page to specify how the result files will be named for the results of a run, and where the result file will be saved.



Method editor: test20

Result name
Serial no is added to all result names

○ First six letters of method name

● Date

○ Name:

Result directory

Path: Home

Scouting Directory:

__Figure 5-36.__ The Result page in Run setup.

The result file name is constructed by adding a 2-digit serial number to one of the base options listed below. The serial number is incremented automatically each time the method is run.

••the first 6 characters in the method name plus a 2-digit serial number

••the date of the run (in a 6-digit format determined by the country setting in OS/2) plus a 2-digit serial number

••a freely specified name of up to 6 characters (within the file naming restrictions in the operating system) plus a 2-digit serial number

If the result file directory already contains files with the same file name base, the serial number is incremented automatically.

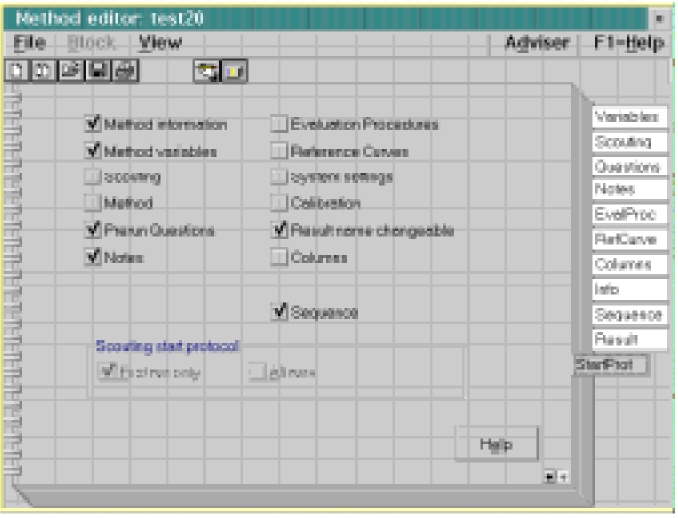
By default, result files are stored in the home directory of the user who starts the run. To change the directory where the result file will be stored, press the **Set path** button, double-click on the required directory icon and press **Exit.**

Note: The result name may be specified as changeable in the start protocol (see Section 5.6.8). In that case, the specification in the **Result** page serves to generate the *suggested* result name, which may be changed at the start of the run.

The Scouting Directory box is not relevant for UNICORN OS.

5.6.8 Start protocol

The start protocol determines which items of the Run setup are displayed (and may in appropriate cases be altered) at the start of a run. Open the **StartProt** page and check the items that are to be displayed.



☑ Method information
☑ Method variables
□ Scouting
□ Method
☑ Prerun Questions
☑ Notes
□ Evaluation Procedures
□ Reference Curves
□ System settings
□ Calibration
☑ Result name changeable
□ Columns
☑ Sequence

Scouting start protocol
☑ First run only
□ All runs

Figure 5-37. *The StartProt page in Run setup.*

Options that are most relevant for UNICORN OS are as follows:

Method information

If this box is checked, the method information (including creator, target system, and date and time of creation and latest change) will be displayed at the start of the run. You cannot edit the method information.

Method variables

If this box is checked, values for method variables will be displayed and can be changed at the start of the run. These values will override the default values for the particular run, and will be recorded in the result file. The defaults stored in the method are however not affected. Variables defined for scouting cannot be changed in the **Variables** page.

If the **Variables** box is not checked, the run will be executed with default values for all variables as defined in the method.

Method
Displays the method instructions. Double-click on a blue Call instruction to display or hide the instructions in the called block Method instructions cannot be changed from this display.

Prerun Questions
If this box is checked, questions defined in the method will be displayed at the start of the run.

Important!
If the **Questions** box is not checked, questions will not be displayed even if they are defined as mandatory. Since the answers to questions can form an important part of the run documentation, you are recommended always to check the **Questions** box.

Notes
If this box is checked, the notes page will be displayed at the start of the run. You can enter notes in the **Start notes** field but not in any of the other fields. You can use the scroll bar if necessary to read notes in the **Method notes** field.

The start of the run is the only occasion when you can enter start notes. If the **Notes** box is not checked, the notes will not be displayed and you cannot enter start notes for the run.

Evaluation procedures
If this box is checked, the evaluation procedures set to execute at the end of the method will be displayed at the start of the run. You can change the choice of procedures to execute, but you cannot add or remove procedures. (Procedures are stored as part of the method file, which cannot be changed at the start of the run).

If the **Procedures** box is not checked, the procedure settings saved in the method will apply at the end of the method.

System settings
If this box is checked, the system settings (including alarms, monitors and curve configuration) will be displayed for information at the start of the run.

To change system settings, use the **System:Settings** command in the System control menu before starting the run (see Chapter 13).

Result name changeable
If this box is checked, you can change the result name when the run is started. Click on the **Set** button to change the result directory.

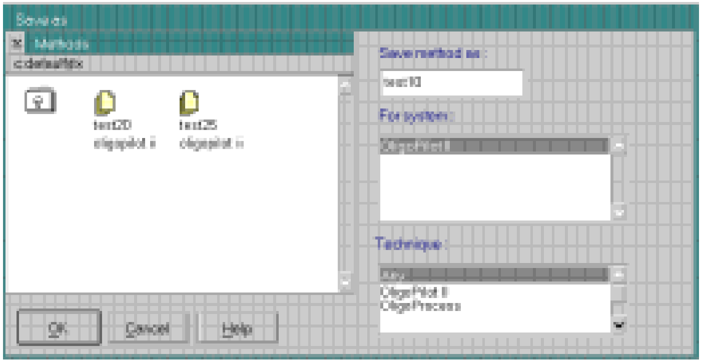
If the **Result name changeable** box is not checked, the result name will still be displayed, but neither the name nor the directory can be changed.

Sequence
This option should be selected to display the sequence that the method has been created for. This is for information only.

5.7 Saving the method

5.7.1 Saving as a method

When you click on the **Create** button in the Sequence editor page, UNICORN OS generates the method, updates the method variables and automatically opens the **Save As** dialogue box. The method must be saved before it can be run.



Save method as : test10

For system : OligoPilot II

Technique : Any

Figure 5-38. *Dialogue box for saving a method. The Methods panel will be shown in icon or detail view according to the current setting in the Main menu. You can change the display mode from the pull-down menu, activated by clicking with the right mouse button in the panel (see Section 3.2.3).*

1. Enter a name for the method. Method names may be up to 8 characters long and may contain letters (A-Z), digits (0-9) and underscore characters. The case of letters is not significant. The method name must be unique for the chosen system within the directory (see steps 2 and 3 below).

2. By default, the method will be saved in your home directory. To change the directory, double-click on the appropriate directory icon in the **Methods** panel.

3. If you have more than one system available, choose the system for which the method is intended. The method can only be run on the system for which it is saved. Remember that different systems may have different configurations and control capabilities.

4. Click on **OK**.

Save

The method remains open in the Method editor when it has been saved, so that you can continue editing if you wish. If you edit any of the method instructions or change the method variables, you can save these changes by selecting **File:Save** or clicking on the **Save** button in the toolbar. If you want to save a copy of the method under a new name, choose **File:Save As** and enter the details as described above. If you close an open method or the Method editor module and there are unsaved changes, you will be prompted to save these changes.

5.7.2 Saving as a template

You can save the method as a template if you have **Edit global lists** authorization.

1. Choose **File:Save as template**.

2. Enter a name for the template in the **Name** field, or choose an existing template name from the list. If you choose an existing name your will overwrite the existing template.

3. Choose the system for which the template is intended in the **For system** field.

4. Click on **OK**.

The templates for each system are common for all users. Be restrictive in saving methods as templates. We recommend that only methods that are useful for all users are saved as templates.

5.7.3 Deleting a template

You can delete templates if you have **Save templates and global evaluation procedures** authorization.

1. Choose **File:Delete template**.

2. Select the system and the template to delete, and click on **OK.**

Note: The templates for each system are common for all users. Be restrictive in deleting templates.

5.8 How to use selected method instructions

This section provides recommendations for how to use some common programming features in UNICORN OS methods.

5.8.1 BASE and METHODBASE instructions

Every method block must start with a BASE instruction, defining the base for calculating breakpoints.

Note: Do not confuse the concept of a *method base* instruction with the bases in a sequence.

The method base may be volume (ml or l depending on the scale defined in the system strategy), time (min), column volume CV (defined as a numerical value or taken from the column definition). For all blocks other than the main block, the base may also be defined as SameAsMain, which means that the block will inherit the base defined in the main block. Different blocks may use different bases.

Use the method base which most closely suits the purpose of the block.

Be careful when changing the base for an existing method. Changing between time and volume base can affect the relative duration of steps in the method if different steps use different flow rates.

Note: For method blocks which use a volume or column volume base, make sure that the flow rate is not zero. Volume breakpoints are calculated from the flow rate of the pump, and the method will not progress if the flow rate is zero.

The parameters for the BASE instruction differ slightly according to whether a named column definition is used.

5.8.2 Instructions at the same breakpoint

Instructions placed at the same breakpoint in a block are executed simultaneously, with the exception of successive CALL instructions which are executed in the sequence in which they are written. This can have important consequences in some situations. For example, the instruction sequence:

```
...
0.00   Base SameAsMain
0.00   WasteOut Waste_ACN
0.00   PFlow_ACN 2.00{bar}
(10.00)#CV_Column_wash End_block
...
```

will set the waste valve to Waste ACN at the same time as 10 column volumes of ACN are pumped through at a pressure of 2.00 bar. Conversely, in the instruction fragment:

```
...
0.00   Call Normal, Detritylation
0.00   Call Normal, Detrit_wash
0.00   Call Normal, Coupling_recycle_DNA_T
       ...
```

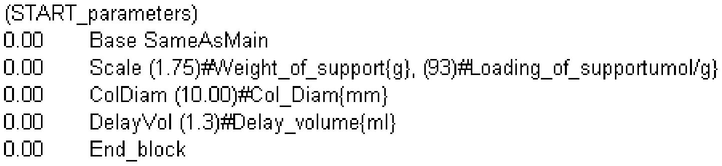
the instructions contained in the first listed call to a block will be completed *before* proceeding onto the next instruction or call to block.

To ensure that instructions are executed in a defined sequence where this is important, separate the instruction breakpoints by 0.1 base units. The revised formulation for the first example above is:

```
...
0.00   Base SameAsMain
0.10   WasteOut Waste_ACN
0.20   PFlow_ACN 2.00{bar}
(10.00)#CV_Column_wash End_block
...
```

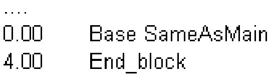
5.8.3 Controlling block and method length

The length of a block is determined by the breakpoint of the last instruction in the block. A block in which all breakpoints are at 0 will take no time or volume during a run, e.g.



```
(START_parameters)
0.00     Base SameAsMain
0.00     Scale (1.75)#Weight_of_support{g}, (93)#Loading_of_supportumol/g}
0.00     ColDiam (10.00)#Col_Diam{mm}
0.00     DelayVol (1.3)#Delay_volume{ml}
0.00     End_block
```

To extend the length of a block without performing any other operation, set the breakpoint of the END_BLOCK instruction appropriately, e.g.



```
....
0.00     Base SameAsMain
4.00     End_block
```

During a run, the overall time or volume is determined by the sum of the block lengths. Note in particular that the length of the main block does *not* indicate the overall length of the method (the main block often consists only of calls to other blocks and has zero length). The accumulated time and volume are displayed in the System control window during a run.

Accumulated time/vol	**Block time/vol**	Instruction
		`(Main)`
0	0	`0.00 Call Normal, Purge_G`
0	0	`    0.00 Base Volume`
0	0	`    . . .`
1	1	`    1.00 End_block`
1	0	`0.00 Call Normal, Purge_C`
1	0	`    0.00 Base Volume`
1	0	`    . . .`
2	1	`    1.00 End_block`
2	0	`0.00 Call Normal, Purge_A`
2	0	`    0.00 Base Volume`
2	0	`    . . .`
3	1	`    1.00 End_block`

Table illustrating the relationship between accumulated and block time/volume for a simplified method fragment.

Depending on how conditional calls are used (see Section 5.8.8 ??), the overall method time or volume may vary according to watch events during the run.

5.8.4 Messages

Use messages to inform the operator of the progress of the run. It is a good idea to issue messages at critical points in the method. The example block below instructs the operator to fill the column with DNA-T support:

```
(Main)
0.00 Base CV, (6.30)#Column_volume{ml}, Any
0.00 Message "Fill your column with DNA-T
     support",Screen
0.00 Message “Press CONTINUE when ready”, Screen
0.00 . . .
```

Messages which are set to Screen will be displayed on the screen during a run, and will remain there until acknowledged by the operator. Messages can also be set to Noscreen: these will be recorded in the run log but not displayed on the screen.

5.8.5 Pausing a method

A method can be programmed to pause at critical points. There are two instructions for this purpose:

Hold Suspends execution of the method, but continues to pump eluent at the current flow rate and concentration settings.

Pause Suspends execution of the method and stops the pumps so that the system comes to a standstill. In OligoPilot II, valves remain in the position they were in before the pause. The pause may be defined as indefinite or for a given number of minutes. This instruction is most useful for stopping the system in the event of an unexpected condition.

In both cases, the method may be resumed by pressing the **Cont** button in the System control toolbar (see Section 6.2).

Note: Never select **Pause** or **Continue** during a system **Hold** (Vol_amid, Vol_Ox, Vol_Thio, Vol_Cap), as this will interrupt the adding of reagent.

5.8.6 Linear flow rates

Linear flow rates (cm/h) for ACN, detrit solution and reagent can be specified by the instructions LFlow_ACN, LFlow_Det and LFlow_Reag respectively. To use these instructions, it is necessary for a column diameter to be defined in the Variables page of Run setup. The volume flow rate is calculated from the specified linear flow rate and the column diameter. The calculated volumetric flow rate is shown during runs.

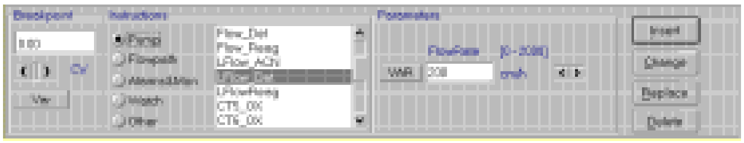


Figure 5-39. *Setting linear flow rate in a method block. The linear flow rate option is only available if the column diameter is defined in the method.*

Note: If a column diameter has not been defined in the method, linear flow will not be able to be used.

5.8.7 Conditional instructions

Conditional (WATCH) instructions allow the progress of a run to be determined by the events during the run, e.g. wash steps to be continued until a specific conductivity has been reached. The system strategy includes a WATCH instruction for each monitor defined in the system. A watch is active from the point at which it is issued until either:

••the watch condition is met

••a new watch is set for the same monitor

••a WATCH_OFF instruction is issued for the monitor

Watch conditions
The conditions for which a watch can be set are as follows for most monitors:

Greater_than The signal exceeds a specified value.

Less_than The signal falls below a specified value.

Slope_greater_than The rate of change of the signal exceeds a specified value, expressed in monitor units/minute. The slope is calculated from the average signal over a number of data points between 1 and 10 set when the system is configured.

Slope_less_than The rate of change of the signal falls below a specified value, expressed in monitor units/minute. The slope is calculated from the average signal over a number of data points between 1 and 10 set when the system is configured.

Less_than_or_valley The signal falls below a specified value or a valley is detected. A valley is defined by a local minimum followed by an increase to 102% of the local minimum value plus the Min_peak value (see below).

Peak_max The signal falls to a specified fraction of the most recent peak maximum minus the Min_peak value (see below). Factor=1 detects peak maximum.

Stable_baseline The signal is stable within the limits of the D_Baseline value (see below) for the period specified by the minutes parameter.

Int_Status Equal to 0 or 1 to indicate if peak integration is switched off or on respectively.

To determine suitable values for watch conditions, it is often most convenient to examine data from a test run. For slope values, use the **Differentiate** function in the Evaluation module to measure the slope of the test chromatogram.

Note: The slope criteria operate on the arithmetic value of the slope, so that a value of -3 is *less* than a value of -2. The end of a peak is thus detected by Slope_greater_than with a negative value (the slope is negative but increasing).

Two conditions apply for air sensors (not available for OligoPilot II):

Equal 0	Air is not detected by the sensor.
Equal 1	Air is detected by the sensor.

Note: To use the WATCH_AS instruction for air sensors, the ASALARM setting in **Alarms&Mon** must be disabled (use the Method editor to disable the alarm locally in a method, or the system settings to disable the alarm for all methods, see Section 13.1). The ASALARM setting overrides any WATCH_AS instruction, and if the alarm is enabled the method will pause when air is detected.

System settings for watch conditions
For continuous monitoring, the following parameters in the **Peak"Monitor"** instruction (e.g. **PeakCond1**) in system settings affect the interpretation of certain watch conditions:

MinPeak
The **MinPeak** setting affects only the Less_than_or_valley and Peak_max conditions. This setting:

(a) sets the threshold for signal increase after a local minimum which will be interpreted as a valley for the Less_than_or_valley condition. A valley and a new peak are detected when the signal increases to 102% of the local minimum plus the MinPeak value.

(b) sets the threshold for signal decrease after a local maximum which will activate the Peak_max condition. Peak_max is detected when the signal falls to the specified fraction of the most recent peak maximum minus the Min_Peak value.

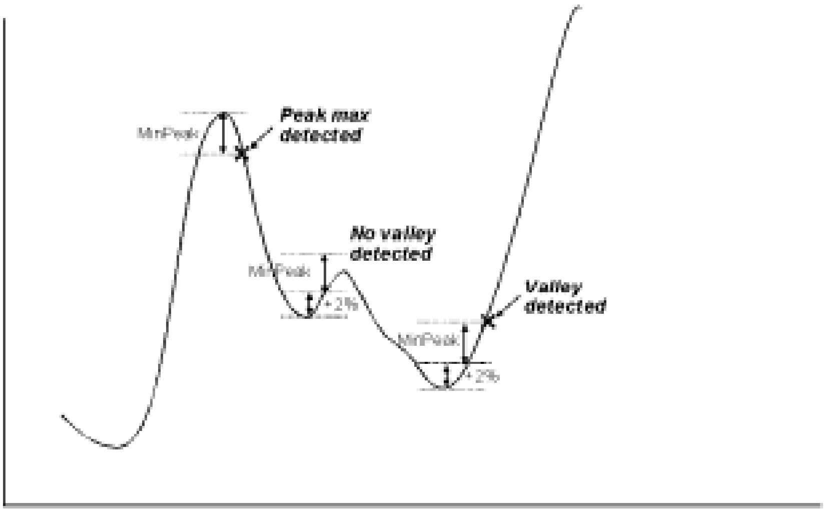


__Figure 5-40.__ Illustration of the MinPeak setting. Peak max is detected when the signal falls by MinPeak from a local maximum if the Peak_max factor is set to 1. Valleys are detected when the signal rises by 2% of a local minimum value plus MinPeak value.

The MinPeak value should be set large enough so that signal noise does not activate the conditions, and small enough so that the condition is activated close to the valley or peak. As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Setting a value that is too high can prevent a new peak from being detected after a local minimum.

D_Baseline

D_Baseline defines the permitted variation for the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the D_Baseline value up or down over the time interval specified in the Stable_baseline condition in the watch instruction.

The D_Baseline setting affects only the Stable_baseline condition.

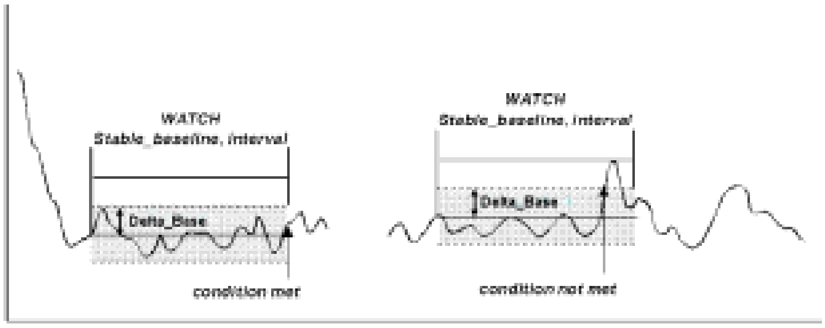


Figure 5-41. *Illustration of the D_Baseline setting. The condition WATCH Stable_baseline is met if the signal does not deviate by more than ±D_Baseline from baseline during the time interval specified for the watch. The baseline value is determined by the signal at the start of the watch. If the condition is not met, a new interval is started with a new baseline value defined by the signal level at the start of the new interval.*

Watch actions
The following actions can be taken when a watch condition is met:

Block name	Call the named block. The call is always made in Normal mode (see Section 5.3.2)
Pause, Hold	Pause or hold the method
Continue	Continue the method if paused or held
End_block	End the current block and return to the point from which the block was called
End_method	End the method

5.9 Creating methods “from scratch”

This is the most advanced level for creating methods, allowing you to create methods from the very beginning in an empty Main block, without the benefit of an established method template nor the sequence editor interface in the Sequence page of Run setup.

A method is built up entirely using the Instruction Box and Text instructions in the Method editor. All blocks for the entire method must be created and breakpoints, instructions and variables inserted into the blocks.

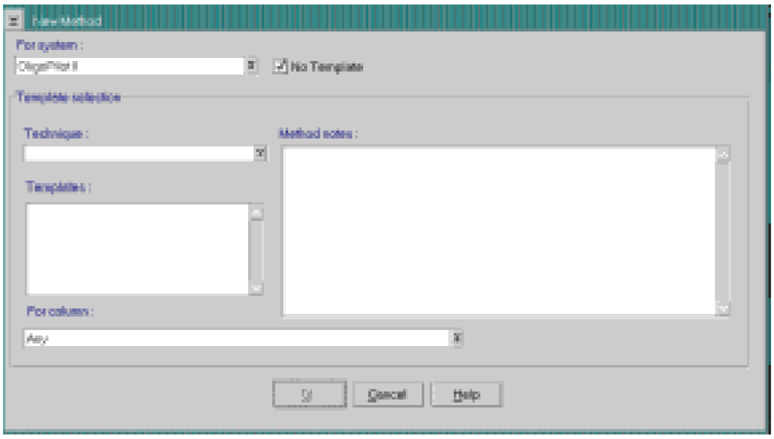
Relevant information must then be inserted into the appropriate pages of Run setup, such as Variables, Questions, Notes, EvalProc, Results and StartProt.

The method can then be saved and run from System control as described in Chapter 6.

There is no real need to use the Sequence page in Run setup since, in creating a method “from scratch”, you have bypassed the need for a sequence editor interface to create methods. If, however, you intend to save the created method as a template for future use with the sequence editor, then you must manually create all of the relevant cross references between the bases and options used in the method to the created blocks.

To create your own method “from scratch”:

1. Click on the **New Method** toolbar button or choose the **File:New:Method** menu command in the Method editor, or choose **File:New:Method** or **New:Method** in the Main menu. These alternatives are equivalent. When you choose the command from the Main menu, the Method editor is opened automatically.



For system : OligoPilot II

☑ No Template

For column : Any

__Figure 5-42.__ The New method dialogue box.

2. Choose the system for which the method is intended.
   The instructions available for a given system are determined by the system strategy. A method developed for one system may not be valid on another.

3. Place a check mark in the **No Template** box.

4. Click on **OK** once you have made your selections. An untitled, empty template, containing only a Main block with a Base instruction, will be opened.

5. Select **View:Text** instructions or click on the **Text** instruction**s** button.

6. Click on **View:Windows** and select **Text** and **Instruction Box** from the list. Click on **OK**.

7. Create a method using the information described in Sections 5.2 to 5.6. Information about the specific instructions are listed in Appendix B.

8. If necessary, create the necessary cross references in the Sequence page of Run setup as described in Section 5.1.

9. Save the method as written or as a method template, as described in Section 5.7.

10. Run the method as described in Chapter 6.

6. Performing a run

This chapter describes how to perform and monitor a run from the System control window. It is recommended that for your first run you use the 20 base pair sequence already supplied with the **fixrec** method template and which you should have saved as a method called **test20** (See Section 4.2). This and other methods that you have created should be present in the **Methods** box of the Main Menu window.

6.1 Starting a method

You can only start a method if the system is connected and no method is currently running. You must have **Run method** authorization on the appropriate system to start a method.

Before starting a method, make sure that:

- ••The correct system is connected in control mode (see Section 6.5). The name of the connected system is shown in the title bar of the System control window, and the connection status is shown on the toolbar. If the correct system is not connected, open a disconnected System control window (or disconnect the system from an occupied window) and choose **System:Connect**. If you cannot establish a control mode connection because the system is controlled or locked by another user, you cannot start a method on the system.

- ••The system monitors are correctly calibrated (see Section 6.6). Calibrating system monitors requires **Calibrate/tune** authorization in the user profile (see Section 12.2).

6.1.1 Starting from the Main menu

You start a method from the Main menu by selecting the method in the Methods panel and choosing **Run** from the pull-down file menu or clicking on the **Run** button.

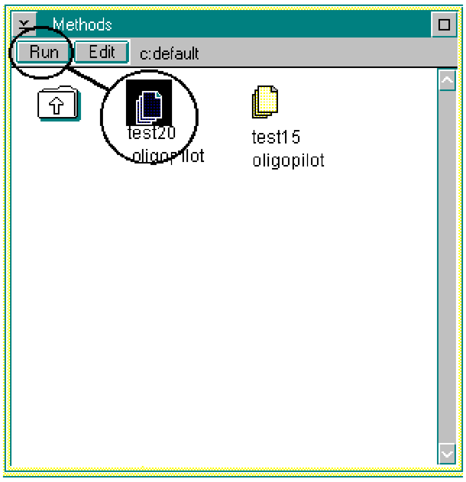


Figure 6-1. Starting a method from the Main menu.

Do not double-click on the method as this will open the Method editor with the method loaded.

6.1.2 Starting from System control

You can also start a method from the System control window. Double-click on the System control icon at the bottom of the screen. If you have more than one system installed, make sure you open the System control window for the correct system.

From the System control window, choose **File:Run**. Double-click on the method icon in the resulting dialogue box, or select the icon and click on **Run**.

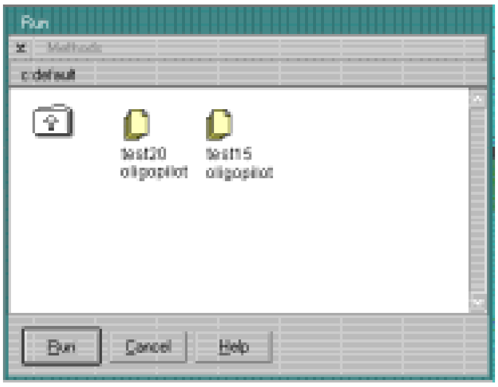


Figure 6-2. Starting a method from the System control menu.

For methods which are used frequently it may be convenient to define the methods as commands in the **File** menu. To do this, choose **File:Menu** and select the required method. The method name will appear as a command in the **File** menu, and choosing the command will start the method.

6.1.3 Start protocol

Methods should be defined with a start protocol which will be displayed before the method actually starts. Work through the start protocol, answering questions as required. As each screen is completed, click on **Next** to move to the next screen or **Prev.** to return to the previous screen. The last screen has a **Start** button to start the method. At any stage, click on **Cancel** to abort the method start.

You are recommended to include the following start protocol items in the display (see Section 5.6.8 for more details):

Method Variables All the variables defined in the method instructions, organized by block. Values for variables can be entered or changed here for the current run.

Prerun Questions Questions are data entry fields which are filled in by the operator when the run is started. Some questions may be mandatory and some may require authorized confirmation.

Notes Method notes are displayed and start notes can be entered.

Method Information Information about the method being run.

Oligo Synthesis sequence The sequence of oligonucleotide to be synthesized. This cannot be altered.

Result Name Changeable The name of the result file is specified here. This page is always displayed, and the name may be changed if this is permitted in the start protocol.

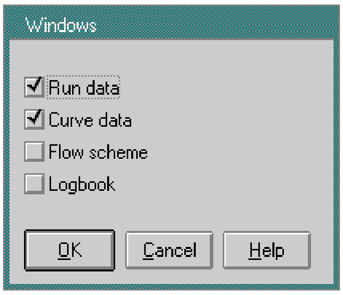
If any questions in the start protocol require authorized confirmation, you will be asked for a username and password when you attempt to leave the screen containing the questions. Only users with **Confirm, Unlock** authorization may authorize answers to such questions. One authorization is valid for all questions in a single screen.

6.2 Monitoring a run

The System control window displays the status of the current system. There may be up to four System control windows available on UNICORN OS desktop which may be connected to different systems. Icons are identified either as **Disconnected!** or by the name of the connected system. Separate systems may be controlled and displayed independently of each other.

Windows

Each System control window displays up to four panels for monitoring different aspects of the run. Click on the **Windows** toolbar button or choose **View:Windows** from the menu to select which panels to display.



Windows

☑ Run data
☑ Curve data
□ Flow scheme
□ Logbook

Figure 6-3. *Dialogue box for choosing panels to display in the System control window.*

6.2.1 General panel techniques

Panels in the System control window are always displayed at the full width of the System control window. Horizontal yellow lines separate multiple panels in the same window. You can drag these lines with the mouse to change the vertical size of the panels.

Hide panel

The **Hide panel** button in the top left-hand corner of each panel closes the panel.

/ **Maximize/Restore**

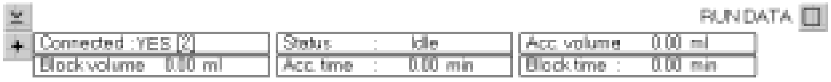
The **Maximize/Restore** button toggles the panel size between adjustable and full window. A panel which is maximized will hide any other panels.

Contents

The **Contents** buttons in the run data and curves panels open the **Run data contents** and **Curve contents** dialogue boxes respectively (see below).

6.2.2 Run data

The run data panel displays the current values for selected run parameters. Values are updated at least every 5 seconds (the actual interval is defined in the system strategy).



RUN DATA

Connected : YES

Status : Idle

Acc. volume 0.00 ml

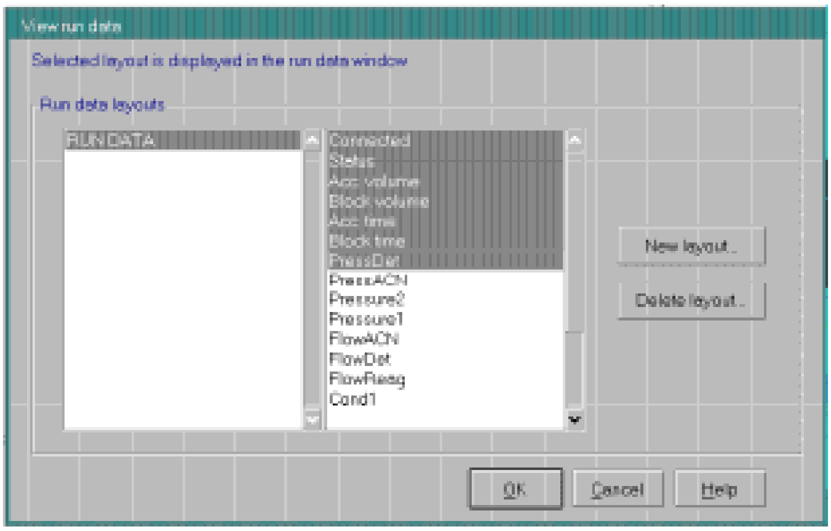
Block volume 0.00 ml

Acc. time : 0.00 min

Block time : 0.00 min

__Figure 6-4.__ The run data panel.

Click on the **+** button or choose **View:Run data contents** to open a dialogue box for selecting which parameters to display. The list of available parameters depends on the system strategy.



View run data

Selected layout is displayed in the run data window

Run data layouts

RUN DATA

Connected
Status
Acc. volume
Block volume
Acc. time
Block time
PressDet
PressACN
Pressure2
Pressure1
FlowACN
FlowDet
FlowReag
Cond1

__Figure 6-5.__ The View run data contents dialogue box.

Different run data layouts (each consisting of a subset of the possible run data items) can be defined for convenient switching between different presentations.

To define a run data layout:

1. Select **View:Run data contents** from the System control menu.
2. Click on **New layout** in the dialogue box.
3. Enter a name for the layout and click **OK**.
4. Select the run data items to be included in the layout and click **OK**.

To edit a layout definition, select the named layout in the **View run data** dialogue box and change the selected run data items in the list.

To delete a layout definition, select the named layout in the **View run data** dialogue box and click on **Delete layout**. You will be asked to confirm the delete operation.

You can choose run data items to display without using named layouts, simply be selecting or deselecting items in the list in the **View run data** dialogue box. Note, however, that this will change the definition of the currently selected layout.

6.2.3 Curves

Displays monitor signal values graphically. Click on the **+** button on the left of the panel or choose **View:Curve contents** to open a list of available curves. The curves in this list are those for which STORE is set to ON in the system settings (see Section 13.4) together with any reference curves defined in the method.

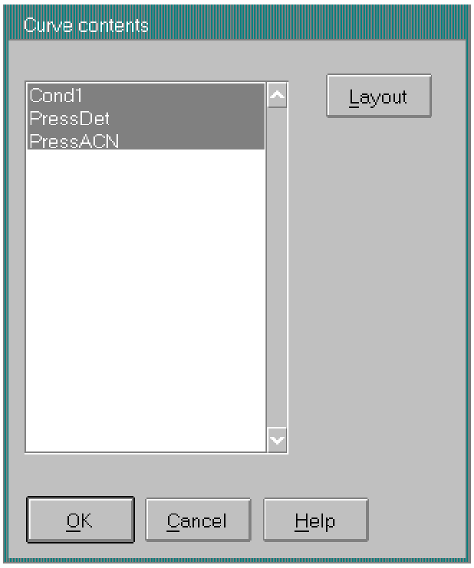
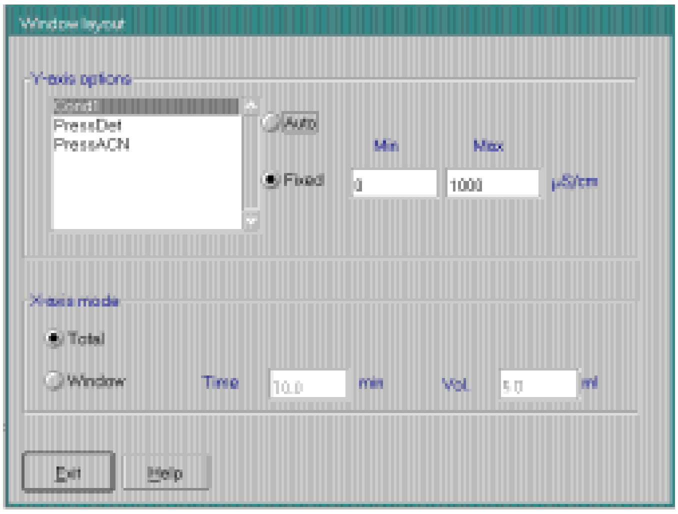


Figure 6-6. *The Curve contents dialogue box.*

In the curve contents box panel, click on **Layout** to adjust the scales for curve presentation.



Window layout

Y-axis options

Cond1
PressDet
PressACN

○ Auto

● Fixed — Min: 0 — Max: 1000 µS/cm

X-axis mode

● Total

○ Window — Time: 10.0 min — Vol.: 5.0 ml

Figure 6-7. Curve Window layout dialogue box.

The y-axis can be set to either auto mode or to a fixed scale with user-defined maximum and minimum values. In auto mode, the scale is adjusted continually according to the highest and lowest values of the curve in the current run. The y-axis scaling can be set independently for each curve.

The x-axis can be set to show the total run from time 0, or to show a fixed time or volume window. The window stretches back in time from the present moment.

The curves panel displays graphs for the selected curves in different colours, with any reference curves included with the method as dashed lines. Values on the y-axis apply to the curve with the same colour as the axis markings. Click on the y-axis to change the axis markings between the displayed curves. Single-click on the x-axis to switch the display between time and volume units. (The run is controlled according to the time/volume base defined in the current block, regardless of the base in the curves display.)

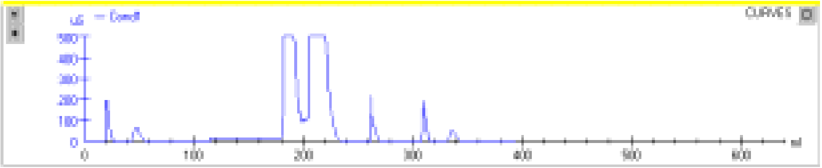
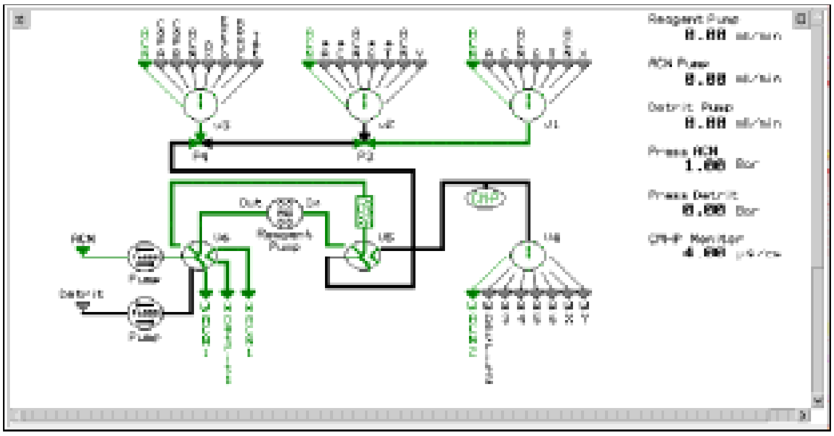


Figure 6-8. The curves panel

6.2.4 Flow scheme

The flow scheme is a graphical representation of the oligonucleotide synthesis system. During a run, the flow scheme displays open flow path(s) in colour and monitor signals with numerical displays. The flow scheme thus shows the current status of the run at a glance.



Reagent Pump
0.00 ml/min

ACN Pump
0.00 ml/min

Detrit Pump
0.00 ml/min

Press ACN
1.00 Bar

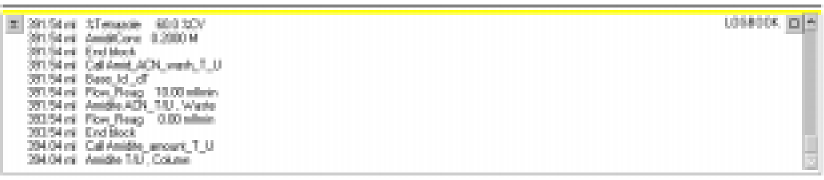
Press Detrit
0.00 Bar

CM-P Monitor
4.00 μS/cm

Figure 6-9. *The flow scheme for a run.*

6.2.5 Logbook

All actions (including method start and end, method instructions and manual interventions such as Pause or Hold) and unexpected conditions such as warnings and alarms are logged for every run, with date, time and current username where appropriate (the date and time are taken from the system clock in the PC). The logbook thus provides complete history of any given run including trityl data, which is a measure of the coupling efficiencies. The log can contain up to 12000 lines and is saved in the result file.



```
391.54 ml  3:Tetrazole    60.0 %CV
391.54 ml  AmiditConc   0.2000 M
391.54 ml  End block
391.54 ml  Call Amid_ACN_wash_T_U
391.54 ml  Base_Id_off
391.54 ml  Flow_Reag   10.00 ml/min
391.54 ml  Amidite.ACN_T/U , Waste
393.54 ml  Flow_Reag    0.00 ml/min
393.54 ml  End Block
394.04 ml  Call Amidite_amount_T_U
394.04 ml  Amidite T/U , Column
```

Figure 6-10. *The logbook panel for a running method.*

6.3 Manual control

6.3.1 The control bar

The control bar at the top of the System control window contains a set of buttons for starting and stopping the run, accessing run notes and locking the system. The current status of a run is indicated by the colour of the status indicator and by the available buttons (buttons which are not available are grey):

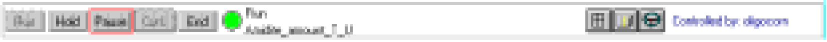


Figure 6-11. *The control bar at the top of the System control window.*

Run Starts a run when the system is idle and a method is loaded.

Hold Suspends execution of a method, but continues to pump liquid at the current flow rate and valve setting. Accumulated time and volume continue to be incremented.
Any method instructions which are set to the time/volume when **Hold** is pressed are executed. Later method instructions are not executed until **Cont.** is pressed.

Pause Suspends execution of a method and stops all pumps so that the system comes to a standstill. In OligoPilot and OligoProcess, the valves remain in the position they were in before the pause. Accumulated time is not incremented during Pause.
Any method instructions which are set to the time/volume when **Pause** is pressed are executed. Later method instructions are not executed until **Cont.** is pressed.

Cont. Resumes execution of a paused or held method.

End Terminates method execution and puts the system in the idle state.

The colour of the status indicator and the available buttons indicate the control status of the connection (buttons which are not available are grey):

Status	**Indicator**	**Available buttons**
Idle	White	Run
Running	Green	Hold, Pause, End
Manual	Green	Run, Pause, End
Hold	Yellow	Pause, Cont., End
Method pause	Red	Hold, Cont., End
Manual pause	Red	Run, Cont, End

Other buttons on the control bar are:

Opens a dialogue box for choosing which window panels to display. Clicking on this button is equivalent to choosing the menu command **View:Window**.

Opens the run set-up pages. Run notes may be entered in the **Notes** page and scouting settings may be changed for scouting run that have not yet started. Other pages are displayed for information only.

The connection mode button has three states which indicate and change the connection mode as described in the table below.

Button	*Connection mode*	*Click to change mode*
	System control window disconnected	Click to connect to a system
☸	Control mode connection	Click to leave system
👁	View mode connection	Click to establish a control mode connection (if possible)

The control bar also displays a text message indicating the connection status of the window:

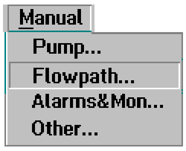
Controlled by :<user> The indicated user has a control mode connection to the system. Other users may establish a view mode connection.

Locked by: <user> The indicated user has left the system in s a locked state. Users who can supply the required password may unlock the system and establish a connection.

System is available Any user may establish a connection.

System connections are described in more detail in Section 6.5.

6.3.2 Manual instructions



The oligonucleotide synthesis system can be controlled with manual instructions (see Appendix B) issued from the **Manual** menu. To save the results of a manual run, issue the instruction RECORD ON (in the **Other** instruction group) at the beginning of the run. UNICORN OS will prompt for a result file name at the beginning of the run.

The **Manual** menu opens a dialogue box similar to the text instruction box in the Method editor (see Section 5.4):

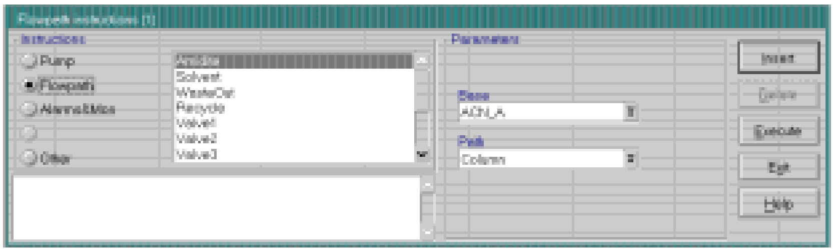


Figure 6-12. *The manual instruction box.*

The number in parentheses in the title bar of the dialogue box identifies the System control window to which the box applies. Available instructions are determined by the strategy for the connected system. Instructions for OligoPilot and OligoProcess strategies are listed in Appendix B.

Manual instructions are entered in the same way as method instructions from the dialogue box in the Method editor. The **Insert** button places the current instruction in the list at the bottom left of the dialogue box. Clicking on **Execute** executes all instructions in the list at the same time, or executes the currently marked instruction if the list is empty. Note that although all instructions are executed simultaneously, some may take some time to complete in the liquid handling module.

The **Delete** button deletes selected instruction from the current list. Only one instruction can be deleted at a time.

If you close the box by clicking on the **Exit** button without choosing **Execute**, commands in the list will not be executed and will be deleted from the command list.

Manual instructions can also be issued while a method is running. A manual setting applies until the next method instruction of the same type is executed (e.g. a manual Flow instruction will set the flow rate until the next Flow instruction in the method is executed). Manual instructions that you issue during a method are recorded in the logbook for the method run.

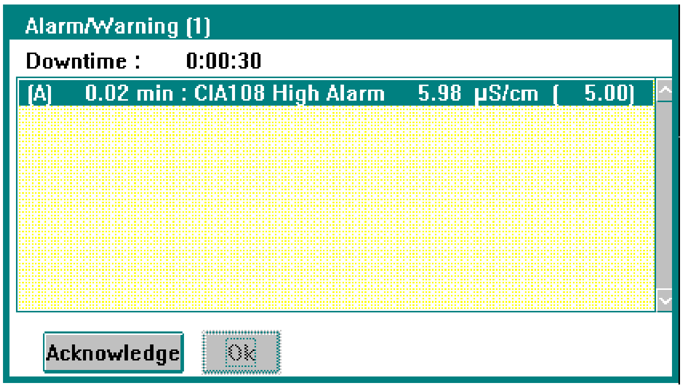
6.3.3 Alarms and warnings

The system settings (see Section 13.1) determine the acceptable limits of monitor signals during a run. The limits can also be set for the current run using an instruction in the method. Limits set with a method instruction override the limits set in system settings. If these limits are exceeded in a run, a warning (W) or alarm (A) box with a message is displayed on the screen.

••The run continues if a warning is issued.

••An alarm pauses the system.

Warnings and alarms are displayed regardless of the activity currently in progress in UNICORN OS: you will be notified of an exceeded limit in a running system even if you are developing a method, evaluating data or monitoring a run on a different system. Warnings and alarms are also recorded in the logbook for the run.



Alarm/Warning (1)

Downtime : 0:00:30

[A] 0.02 min : CIA108 High Alarm 5.98 µS/cm (5.00)

Figure 6-13. *Alarm/warning display box.*

In a network installation, warnings and alarms are only displayed on computers that are connected to the system. An alarm can be acknowledged only from the computer connected in control mode: alarms are displayed but cannot be acknowledged on computers connected in view mode.

Note: For this reason, we discourage “passive” operation of a system, i.e. with no controlling connection.

6.4 If communication fails

This section summarizes the consequences of system failure during a run. See Section 11.3 in UNICORN 2.10 User Manual for more details.

6.4.1 Network failure

If network communication fails during a run that has been started from a remote station, the run will continue and the results will be saved in the FAILED directory on the local station. A control mode connection can be established on the local station to control the running system.

6.4.2 Local station failure

Intermediate results up to the time of the failure are saved if the **Autosave interval** is set to a suitable value in the system definition (see Section 12.1).

6.5 Managing system connections

UNICORN OS installed on a given computer may have up to four System control windows (the actual number is determined when the software is installed (see Chapter 11), each of which may be connected to one oligonucleotide synthesis system at a time. Connections are managed using the **Connect** and **Disconnect** commands in the **System** menu. A network installation may have more than four systems in total, but each computer in the network can establish a maximum of four connections. Connection management is the same for stand-alone and network installations.

6.5.1 Establishing a connection

To connect a System control window to an oligonucleotide synthesis system, open a disconnected window (identified by the text **Disconnected!** below the window icon on the desktop) and choose **System:Connect**. The dialogue box lists the systems to which you have access. Select the system to which you want to connect and click on **OK**.

To connect to a system from a remote station in a network installation, the local station (i.e. the computer physically connected to the oligonucleotide synthesis system) must be logged in to the network. UNICORN OS does not however need to be running on the local station.

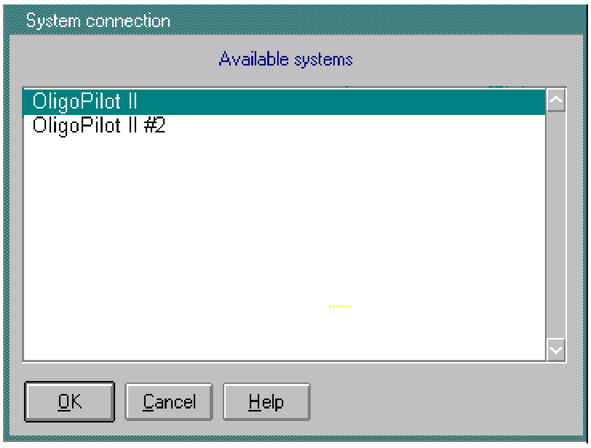


Figure 6-14. *The dialogue box for connecting to a system.*

A local station can be used to control the oligonucleotide synthesis systems directly connected to the PC without logging in to the network. Method and result files stored on network drives will of course not be accessible. For runs performed in this stand-alone mode where the result file is directed to a network drive, the results will be saved in the FAILED directory on the local station (see Section 9.3).

Several simultaneous connections can be established to one system, but only one may be in control mode, i.e. able to actively control the system. The other connections are in view mode, and can monitor the system activity but cannot issue commands (except **Pause** if the user has **Pause** access).

6.5.2 Connection modes

The possible connection states of a System control window are indicated by the connection mode button and the status text on the control bar as summarized below:

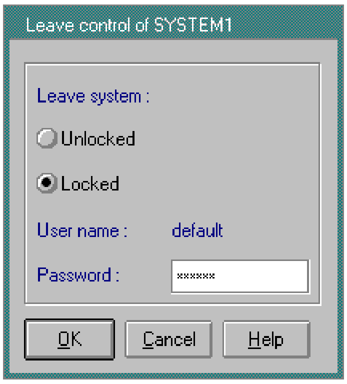
Button	*Connection mode*	*Text*	*State/Action*
🔌	Not connected	(none)	Click on the connection mode button or choose **System:Connect** to establish a connection.
☸	Control mode	**Controlled by: <user>**	Click on the connection mode button to leave the system but retain the connection with the System control window. You may leave the system locked or unlocked.
👁	View mode	**Controlled by: <other user>**	The indicated user has a control mode connection. Clicking on the connection mode button has no effect.
👁	View mode	**Locked by: <other user>**	The indicated user has left the system in a locked state. Click on the connection mode button to establish a control mode connection (you must supply the locking password, or your login password if you have **Unlock locked systems** authorization).
👁	View mode	**System is available**	A user has left the system in an unlocked state. Click on the connection mode button to establish a control mode connection.

In all modes you can choose **System:Disconnect** to disconnect the system from the control window.

6.5.3 Leaving and locking a system

Control mode

A running or idle system with a control mode connection can be left and locked by clicking on the control mode button or selecting **System:Leave system**. When the system is left, the connection becomes a view mode connection. After leaving and locking scouting or sequence runs, it is not possible to establish a control mode connection from another computer.



Leave control of SYSTEM1

Leave system :

○ Unlocked

● Locked

User name : default

Password : ******

Figure 6-15. *The dialogue box for leaving a system.*

You may leave the system unlocked or locked:

••**Unlocked** leaves the system unlocked. Any other user may establish a control mode connection to the system. Use this option if you do not intend to use the system in the near future.

••**Locked** locks the system with the password specified in the dialogue box. A control mode connection can only be established by providing the correct password. Note that this password is independent of the user's login password. A locked system can also be unlocked with the log-in password for a user with **Unlock locked systems** authorization. This authorization should be restricted to a small number of users to prevent indiscriminate unlocking of locked systems.

6.5.4 Disconnecting a system

To disconnect a oligonucleotide synthesis system from a System control window, choose **System:Disconnect**. If you are disconnecting a control mode connection, you will be asked to leave the system first.

Logging out or quitting UNICORN OS automatically disconnects all connected systems, displaying the **Leave control** dialogue box for each system. Systems which are disconnected in this way will be reconnected automatically when you log in to UNICORN OS again.

Note: You may, however, have disconnected from a control mode connection but establish a view mode connection on re-connect, if another user has taken control of the system in the meantime).

Note: You can disconnect a system during a run and the run will continue. It is not recommended to do this without locking the system, since this can leave a run on the system with no responsible user. You cannot however disconnect from scouting or sequence runs.

6.5.5 Network considerations

In a network installation, an oligonucleotide synthesis system can be controlled from any computer in the network provided that the user has sufficient access rights in UNICORN OS. UNICORN OS software has to be installed but not necessarily running on the computer to which the system is physically connected. The computer has to be logged in to the network.

- •A system which is controlled by another user through a control mode connection can be viewed through a view mode connection by any user with sufficient access on any number of computers in the network. This allows runs to be monitored from multiple display stations (although only the active connection can control the system).

- •A system which is locked by a user can be unlocked on any computer in the network by any user with sufficient access rights (see above).

- •A UNICORN OS user may log in to UNICORN OS on any number of computers in the network. Each successive multiple instance of the user automatically establishes the same System control connections as the first instance when the login is performed. Multiple instances are however treated by UNICORN OS as separate users (although they are not distinguished in the System control window display) and only one of the instances may maintain a control mode connection to a system. Multiple instances may also disconnect and connect their System control windows independently of each other once the login is performed.

6.6 Calibrating monitors

Certain system monitors need to be calibrated regularly for correct results. According to the routines established in the laboratory or process department, monitors may be calibrated at pre-set intervals by the system technician, or calibrated as required by the user before each run.

This section describes the calibration procedure for the ACN and Detrit solvent pumps in OligoPilot II. For other synthesis systems, users are recommended to read the relevant system manual for calibration procedures.

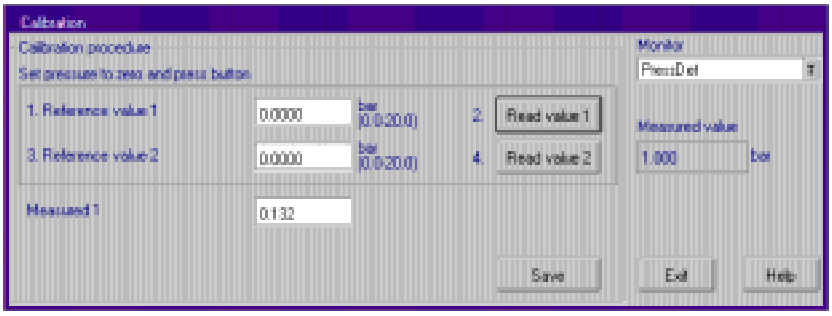
To calibrate the solvent pumps for OligoPilot II do the following:

1. Select **System:Calibrate** to display the **Calibration** box.

2. Select each of the pumps in turn and perform the procedures described below.

3. Click on **Exit** once the calibrations have been made.

Detrit solvent pump



Calibration

Calibration procedure

Set pressure to zero and press button

1. Reference value 1: 0.0000 bar [0.0-20.0]

3. Reference value 2: 0.0000 bar [0.0-20.0]

Measured 1: 0.132

Monitor: PressDet

Measured value: 1.000 bar

Figure 6-16. *Dialogue box for pressure calibration of the detrit pump.*

Select **PressDet** in the **Monitor** pull-down list to calibrate the pressure reading of the Detrit P-6000 solvent pump. The calibration is based on the maximum set pressure of the pump.

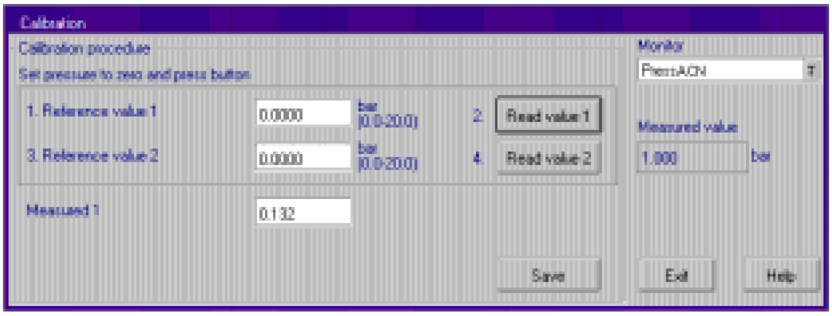
1. The value in the **Reference value 1** box should be zero (0.0000 bar). If not, enter 0.0000. Click on the **Read value 1** button.

2. Enter the desired maximum pressure value (<20.0 bar) in the **Reference value 2** box. On the detrit pump, press and hold the SET button and manually set the pressure to the desired level. While holding the SET button on the pump, you can observe in the UNICORN OS **Calibration** dialogue that an internal **Measured**

value is assigned. Click on the **Read value 2** button and then release the SET button on the pump. Click on **Save** to save the calibration. Refer to the P-6000 Pump Instruction Manual for more detailed instructions.

Note: A value for the actual calibrated pump pressure can be obtained by multiplying the values obtained in the **Measured value** and **Measured 1** fields.

ACN solvent pump



Calibration

Calibration procedure

Set pressure to zero and press button

1. Reference value 1: 0.0000 bar [0.0-20.0]

3. Reference value 2: 0.0000 bar [0.0-20.0]

Measured 1: 0.132

Monitor: PressACN

Measured value: 1.000 bar

Figure 6-17. *Dialogue box for pressure calibration of the ACN pump.*

Select **PressACN** in the **Monitor** pull-down list to calibrate the pressure reading of the ACN P-6000 solvent pump. The calibration is based on the maximum set pressure of the pump.

1. The value in the **Reference value 1** box should be zero (0.0000 bar). If not, enter 0.0000. Click on the **Read value 1** button.

2. Enter the desired maximum pressure value (<20.0 bar) in the **Reference value 2** box. On the ACN pump, press and hold the SET button and manually set the pressure to the desired level. While holding the SET button on the pump, you can observe in the UNICORN OS **Calibration** dialogue that an internal **Measured value** is assigned. Click on the **Read value 2** button and then release the SET button on the pump. Click on **Save** to save the calibration. Refer to the P-6000 Pump Instruction Manual for more detailed instructions.

Note: A value for the actual calibrated pump pressure can be obtained by multiplying the values obtained in the **Measured value** and **Measured 1** fields.

Introductory material

Methods and Runs

Evaluation

System management

Appendices

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7. Presenting results

A result file holds a complete record of a run, including method, system settings, curve data, run log and Trityl data. The Evaluation module offers extensive facilities for presenting and evaluating run data. All of the functions and nomenclature in the Evaluation module of UNICORN OS, are the same as that used for chromatographic separations in UNICORN 2.10. Only those functions relevant to the presentation of synthesis run results is described in this chapter.

This chapter describes how to:

●●view the results from a run
●●present the chromatograms and curves of your result file
●●compare chromatograms and curves
●●print reports

7.1 Opening a result file

To open a result file, double click on a result file icon in the Main menu. Alternatively, click on the Evaluation icon on the Main menu workspace and then select **File:Open** in the Evaluation module. Select the desired result file from the panel that appears. All contents of a result file are transferred to the Evaluation workspace. By default, the first chromatogram in a run is shown as an opened active window. Any other chromatograms are represented by icons on the workspace. Other icons are **username:Temporary** and **Documentation**.

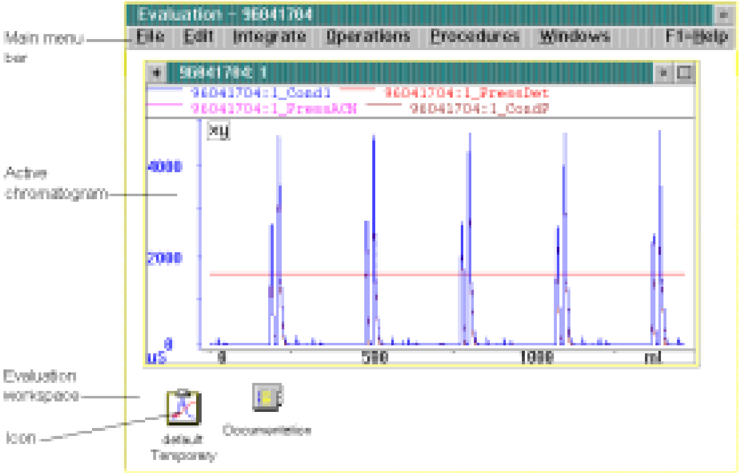
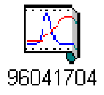
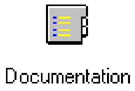
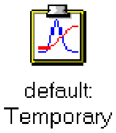


Figure 7-1. *Evaluation module view.*

Icons can be neatly arranged on the workspace by selecting **Windows:Arrange** icons. Icons representing the various components of a result file are as follows:

 96041704	Chromatogram
 Documentation	Documentation
 default: Temporary	Temporary

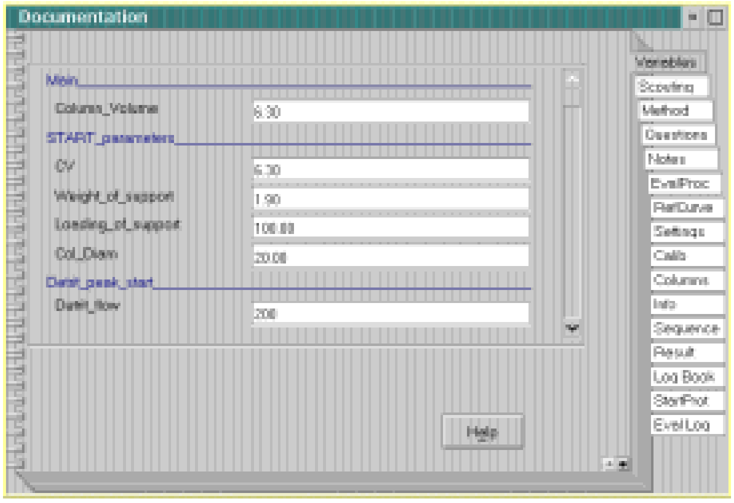
7.1.1 Chromatogram

Curves that have been created during a run, such as the conductivity and pressure of the Detrit and ACN pumps, are stored together in a *chromatogram*. The original raw data curves cannot be deleted or modified, although they can be used as the basis for evaluation procedures and subsequent creation of new curves. A chromatogram also contains the curves created and saved during an evaluation session.

7.1.2 Documentation icon

The full documentation of a run is stored within the result file. The important documentation pages for UNICORN OS are Variables, Method, Questions, Notes, Evaluation procedure, Sequence, Result, Log Book, Start Protocol and Evaluation Log. A few of these are described below. To open the Documentation, either double click on the icon or select **Windows:Documentation**. To print the contents in any of these, select **File:Print** or **File:Report**. The contents can be saved as a new method by selecting the page **Method** and clicking on **Save as**.

Variables
This displays the parameters that were used during the run.



Main

Column_Volume 6.30

START_parameters

CV 6.30

Weight_of_support 1.90

Loading_of_support 108.03

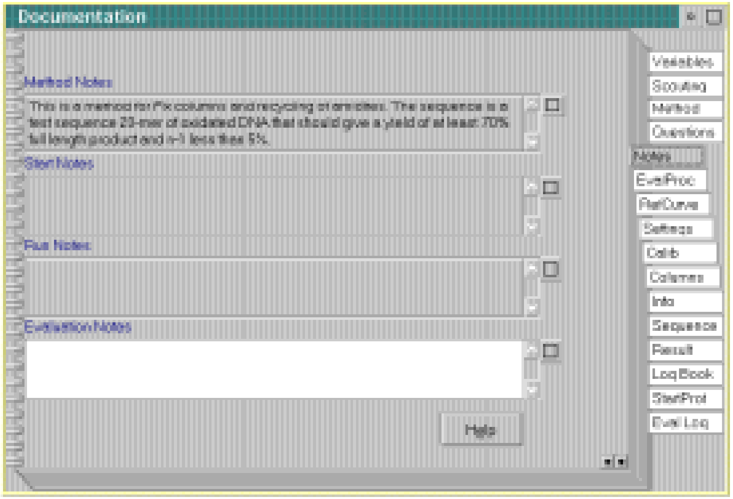
Col_Diam 20.08

Detrit_peak_start

Detrit_flow 200

Figure 7-2. *Variables page.*

Notes
This displays notes that you have made at various points during the run. You are also able to enter new comments in the **Evaluation Notes** field.



Method Notes

This is a method for Fix columns and recycling of amidites. The sequence is a test sequence 23-mer of oxidated DNA that should give a yield of at least 70% full length product and n-1 less than 5%.

Start Notes

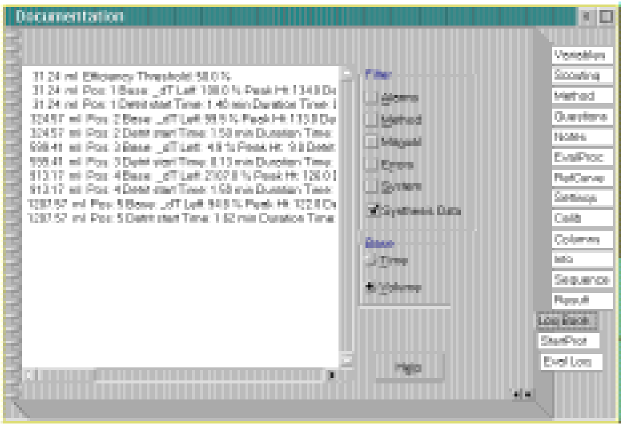
Run Notes

Evaluation Notes

Figure 7-3. *Method Notes page.*

Log book

This displays exactly what happened during a run, including information concerning alarms, the method, manual changes, errors, the system and the oligonucleotide sequence. Selecting (checking) the **Synthesis Data** option provides information about the coupling efficiency of each base addition to the oligonucleotide. Trityl-ON synthesis will not display the last 5´ base in the Trityl table since the last base is not detriylated. Thus, in a synthesis of a 25-mer trityl-ON there will only be 24 Detrit values in the synthesis data. Synthesis data can also be obtained by printing a report (see Section 7.6).



```
31.24 ml Efficiency Threshold: 58.0 %
31.24 ml Pos: 1 Base: _dT Left: 100.0 % Peak Ht: 134.8 De
31.24 ml Pos: 1 Detrit start Time: 1.40 min Duration Time: 
324.57 ml Pos: 2 Base: _dT Left: 98.9 % Peak Ht: 115.8 De
324.57 ml Pos: 2 Detrit start Time: 1.50 min Duration Time
598.41 ml Pos: 3 Base: _dT Left: 4.8 % Peak Ht: 9.8 Detrit
598.41 ml Pos: 3 Detrit start Time: 8.13 min Duration Time
913.17 ml Pos: 4 Base: _dT Left: 2107.8 % Peak Ht: 128.0 
913.17 ml Pos: 4 Detrit start Time: 1.58 min Duration Time
1280.57 ml Pos: 5 Base: _dT Left: 94.8 % Peak Ht: 122.8 De
1280.57 ml Pos: 5 Detrit start Time: 1.62 min Duration Time
```

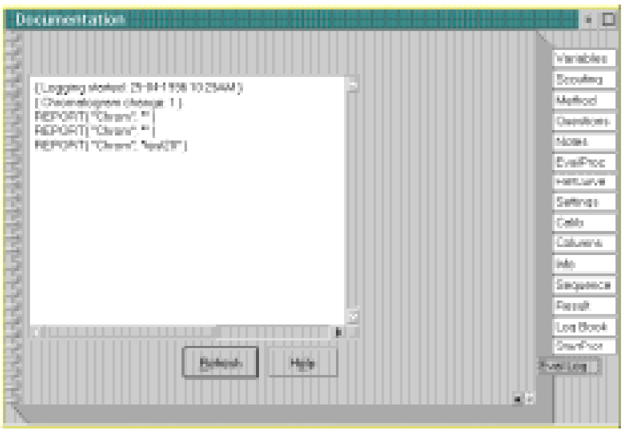
Filter
□ Alarms
□ Method
□ Manual
□ Errors
□ System
☑ Synthesis Data

Base
○ Time
● Volume

Figure 7-4. *Log Book page.*

Evaluation log

This lists all of the evaluation operations performed for the current result file for all sessions, including procedures executed at the end of the method. Click on the **Refresh** button to update the list.



```
{Logging started: 25-04-1998 10:25AM }
{ Chromatogram change: 1 }
REPORT( "Chrom", "" )
REPORT( "Chrom", "" )
REPORT( "Chrom", "test2f" )
```

Figure 7-5. *Evaluation Log page.*

7.1.3 Temporary icon

The **Temporary** icon is specific to the Evaluation module and is essentially an empty chromatogram. Thus, curves can be copied into **Temporary** and comparisons and/or evaluations can be performed. This is particularly useful if you do not want to clutter up your original chromatograms with a large number of curves. Information contained within the **Temporary** chromatogram is automatically saved from one evaluation session to the next, but is not saved within the result files. Double click on the icon or select **Windows:Temporary** to open it. The contents of the temporary chromatogram can be removed by selecting **Edit:Clear temporary chromatogram**.

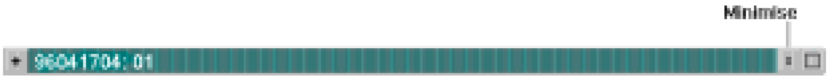
7.2 Basic presentation of chromatograms

This section gives directions on how to access result files and optimize the presentation of a chromatogram and its curves via the **Chromatogram Layout** dialogue box.

7.2.1 Viewing chromatograms

Chromatograms can be selected and made active in two possible ways: either click on the relevant icon on the Evaluation workspace or select the desired chromatogram in the **Windows** menu.

To minimize the active chromatogram to an icon, click on the **Minimize** button in the top-right corner of the window.



What you get to start with

The first time a chromatogram is opened and viewed, a default layout will be applied where all of the original curves are displayed. The default layout can be changed by the user (see Section 7.2.9).

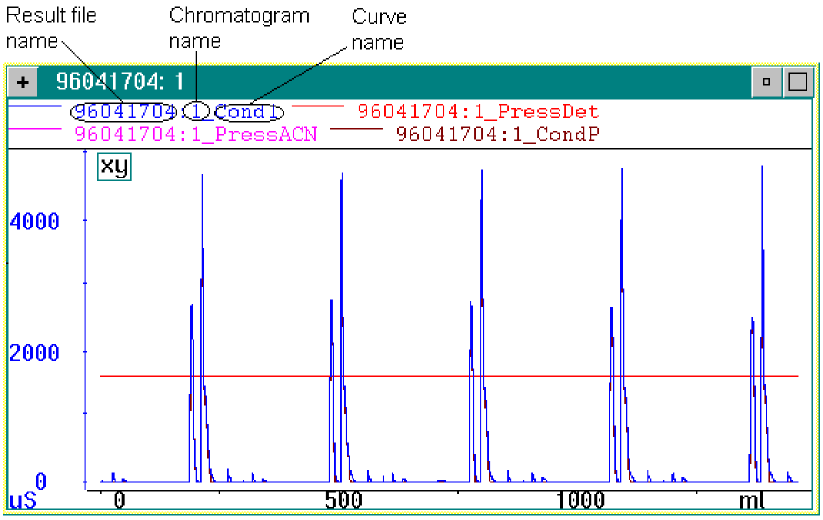


Figure 7-6. Displayed chromatogram in a newly opened result file.

Each curve has its own colour, as denoted by the legends in the chromatogram header. Each curve legend consists of the result file name, chromatogram name and curve name.

Each coloured curve has a correspondingly coloured y-axis. To choose the relevant y-axis scale, click repeatedly on the y-axis until the desired scale is displayed.

By default, the x-axis is shown in volume (ml). To change the x-axis units, see Section 7.2.7.

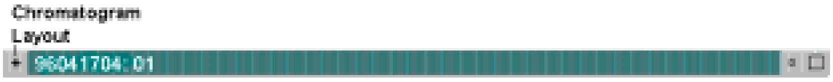
Optimizing the workspace
To maximize the size of an active chromatogram window in the available workspace area, click on the **Maximize** button in the top-right corner of the window.

Alternatively, select **Windows:Tile**. This option is particularly useful when you want to simultaneously view all of the opened chromatograms in the maximum available space.

Selection of **Windows:Cascade** will stack all of the active windows like a deck of cards.

7.2.2 The Chromatogram Layout dialogue box

Most of the changes that you are likely to make regarding chromatogram presentation, are made in the **Chromatogram Layout** dialogue box. This is opened in one of two ways: either click on the command button containing a black cross in the top-left corner of the active chromatogram, or select **Edit:Chromatogram Layout**.



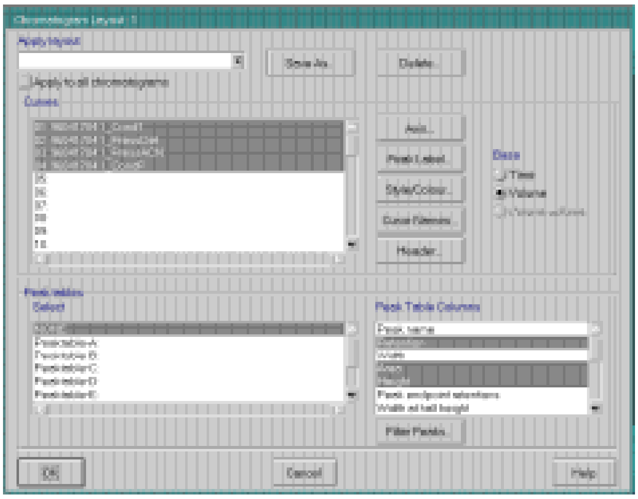


Figure 7-7. *Chromatogram Layout dialogue box.*

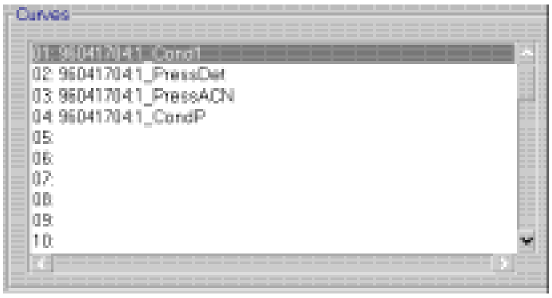
The basic operation of the **Chromatogram layout** box is described in the following sections.

7.2.3 Selecting a layout

If there is a pre-defined layout that you want to apply to the chromatogram, select it from the **Apply layout** pull-down list. If the same layout is to be applied to all chromatograms on the Evaluation workspace select (check) the **Apply to all** option. Click on **OK** to apply the layout. To create pre-defined layouts, see Section 7.2.9.

7.2.4 Choosing the curve(s) you want to see

In the **Curves** field in the **Chromatogram Layout** box is a list of all curves contained within the chromatogram, numbered from **01** onwards. By default, a chromatogram is displayed with all curves presented and, hence, all of the curves in the list are highlighted. Select only those curves you want to see in the chromatogram.



Curves

01: 96041704:1_Cond1
02: 96041704:1_PressDet
03: 96041704:1_PressACN
04: 96041704:1_CondP
05:
06:
07:
08:
09:
10:

__Figure 7-8.__ Curves field in the Chromatogram Layout dialogue box.

Click on **OK** to return to the active chromatogram window.

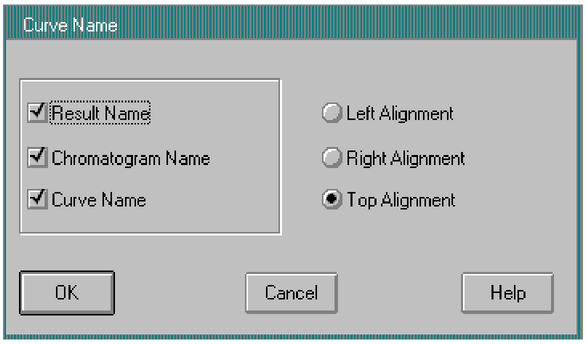
7.2.5 Curve and chromatogram names

By default, each curve legend consists of three components, namely the result name, chromatogram name and curve name (see Figure 7-6). For example, a curve with the name **96041704 : 1_Cond1,** is derived from the result named **96041704.** The chromatogram name is a number automatically given during the run, e.g. **1**. The curve name corresponds to the curve type, e.g. **Cond1** is the conductivity. If two or more curves of the same type were created within a result file, they are numbered sequentially, e.g. **Cond1, Cond2** etc.

Changing the legend name view

If you do not want to display the full legend name in the dialogue boxes and chromatogram windows:

1. Click on the **Curve Names** command button in the **Chromatogram Layout** box.



Curve Name

☑ Result Name
☑ Chromatogram Name
☑ Curve Name

○ Left Alignment
○ Right Alignment
● Top Alignment

__Figure 7-9.__ Curve Name dialogue box.

2. Choose the components of the legend name to be displayed; either **Result name, Chromatogram name** and/or **Curve name**.

3. Choose the display alignment of the names in the active chromatogram window.

4. Click on **OK** to return to the **Chromatogram Layout** box.

It is usually sufficient to display only the curve name if a single chromatogram is being evaluated. However, confusion may arise when more than one chromatogram is shown, so more complete names may be necessary.

Changing the curve or chromatogram name
Sometimes, it may be desired to change the name of a chromatogram or curve. To do this, leave the **Chromatogram Layout** box and then:

1. Select **Edit:Rename**.

2. Select the appropriate option to be renamed; **Chromatogram**, or **Curve**.

3. From the panel that appears, select the appropriate object for renaming and type in the new name. The new name will replace the old one rather than creating a new curve or chromatogram.

Note: The original raw data curves cannot be renamed.

7.2.6 Changing the colour and style of curves

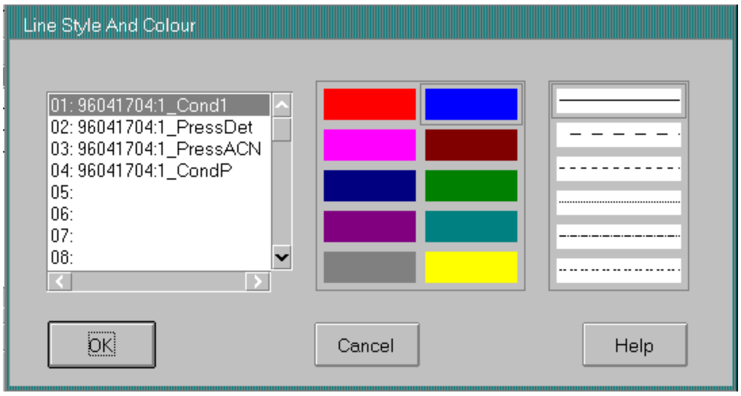
By default, the curves positions within a chromatogram are each represented by a default colour and line style. Curves imported into the chromatogram or newly created curves are thus designated a colour and line style dependent on their position in the curve list.

To reassign the colour and/or line style of a specific curve, do the following:

1. In the **Chromatogram Layout** box, click on the **Style/Colour** button. A panel will appear which shows the colour and line style options.

2. To change the colour and/or line style of a curve, select the curve of interest from the list.

3. Select the desired colour and/or style.



Line Style And Colour

01: 96041704:1_Cond1
02: 96041704:1_PressDet
03: 96041704:1_PressACN
04: 96041704:1_CondP
05:
06:
07:
08:

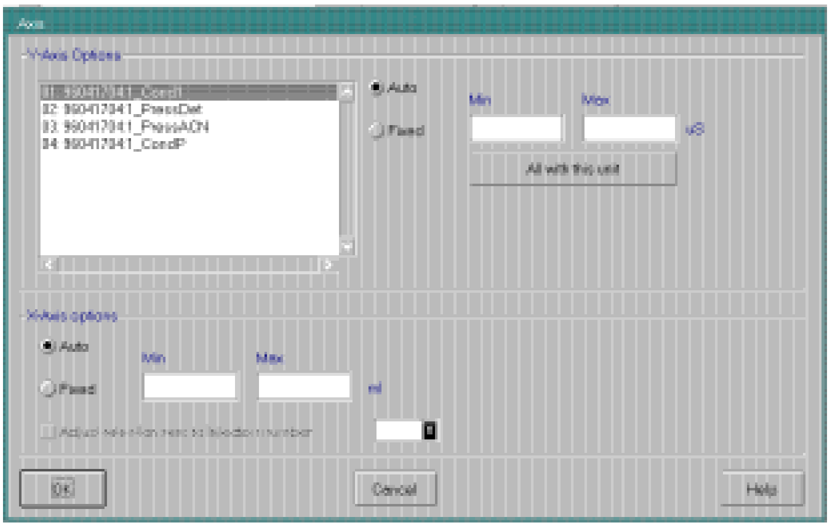
Figure 7-10. *Line Style and Colour dialogue box.*

4. Click on **OK** to return to the **Chromatogram Layout** box.

7.2.7 Changing and fixing the axes

By default, the y-axes are automatically scaled for each curve to show the whole curve. The x-axis is automatically scaled to show the whole run (ml or minutes for OligoPilot and litres or minutes for OligoProcess).

It is possible to 'fix', the minimum and maximum values for the axes of any curve and thereby select a specific part of the curve to be displayed.



Axis

Y-Axis Options

01: 96041704:1_Cond1
02: 96041704:1_PressDet
03: 96041704:1_PressACN
04: 96041704:1_CondP

● Auto
○ Fixed
Min Max uS

X-Axis options

● Auto
○ Fixed
Min Max ml

□ Adjust retention zero to injection number

Figure 7-11. *Axis dialogue box.*

y-axis

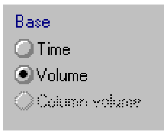
1. Click on the **Axis** command button in the **Chromatogram Layout** box.

2. Select the appropriate curve from the list for which you want to fix the scale. Click on the **Fixed** option.

3. Enter the desired minimum and maximum values for the y-axis. If you click on **All with this unit**, other curves that have the same y-axis units as the current curve, will be similarly scaled. Click on **OK**.

Note: **All with this unit** will only be applied to existing curves. It will not be applied to new curves created after this function was last used. New curves are automatically scaled.

x-axis



Base
○ Time
● Volume
○ Column volume

1. Select the appropriate method base from the **Base** field in the **Chromatogram Layout** box. **Time** of retention, **Volume** or **Column volume** may be selected. The default setting is **Volume**. After certain operations, e.g. integration, the base cannot be changed.

2. Click on the **Axis** command button. For the x-axis, click on the **Fixed** option. Type in the desired minimum and maximum values for the x-axis. Click on **OK**.

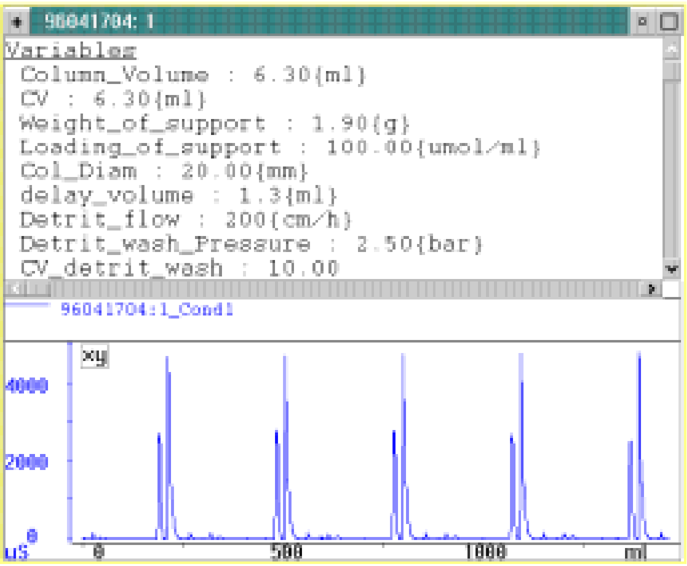
7.2.8 Viewing information about the run

you may wish to display header information at the top of a chromatogram detailing the Variables, Questions and/or Notes. Header information cannot be displayed for imported chromatograms.

1. In the **Chromatogram Layout** box, click on **Header**.

2. A box is displayed that allows you to select any of the four header items **Variables**, **Scouting variables**, **Questions** and/or **Notes**. Click on **OK**.

Note: Scouting variables are not relevant for oligonucleotide synthesis methods.



```
Variables
 Column_Volume : 6.30{ml}
 CV : 6.30{ml}
 Weight_of_support : 1.90{g}
 Loading_of_support : 100.00{umol/ml}
 Col_Diam : 20.00{mm}
 delay_volume : 1.3{ml}
 Detrit_flow : 200{cm/h}
 Detrit_wash_Pressure : 2.50{bar}
 CV_detrit_wash : 10.00
```

__Figure 7-12.__ Chromatogram with header information displayed.

7.2.9 Saving a layout

All changes that you make in the **Chromatogram Layout** box can be saved as a layout. It is possible to apply saved layouts to other chromatograms. All saved layouts are user specific.

Note: A layout saved with the name DEFAULT (in upper-case letters) will be used as the default layout for all chromatograms when they are opened for the first time.

Once a layout has been selected in the **Chromatogram Layout** dialogue box, save it as follows:

Click on the **Save as** command button. Type in the name of your layout and click on **OK**. Several different layouts can be saved in this manner. Layouts are saved for use with other result files. You can now apply your layout(s) as described in Section 7.2.3.

7.3 Additional presentation possibilities

The Evaluation module allows you to perform operations on the curves to optimize the presentation.

Note: The latest evaluation operation that is performed can be undone using **Edit:Undo** or reapplied using **Edit:Redo**.

7.3.1 Showing part of a curve

This section deals with the selection of just part of a curve for purposes of closer examination of details and for presentation. This can be done in three different ways:

- ••magnification using the zoom function
- ••fixing the axes
- ••cutting the curves

The zoom function

In the active chromatogram window, it is possible to zoom-in on a designated area of the chromatogram. This is the easiest and quickest way to magnify different parts of a curve.

1. Place the mouse pointer in any corner of the area to be magnified.

2. Press and hold the left mouse button. A magnifying-glass icon will replace the mouse pointer arrow on the screen.

3. Drag a box from the point of origin to cover the area to be magnified. Release the mouse button.

   The selected region will now be displayed in the entire chromatogram window, together with appropriate scales for the y and x axes.

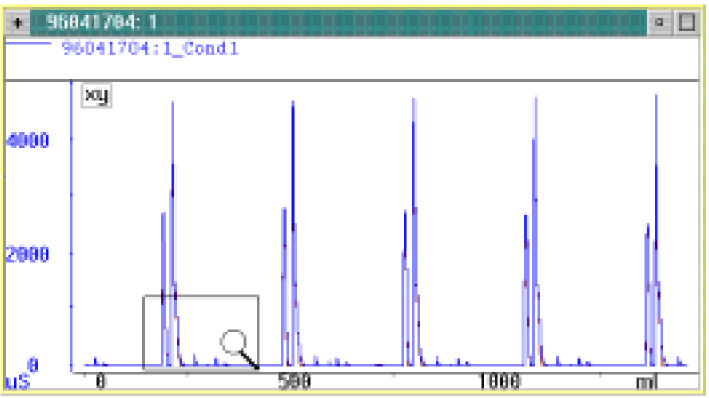


Figure 7-13. *Illustration of the chromatogram zoom function.*

The zoom process can be repeated many times within an already magnified region. You can also use the <PAGE UP> and <PAGE DOWN> keys to zoom-out and zoom-in respectively on the whole chromatogram. You can also use the cursor-arrow keys on the keyboard to move around in the chromatogram at the current magnification scale.

4. Display the original view of the chromatogram by clicking on the **Unzoom** command button in the top-right corner of the active window.

Fixed scale axes

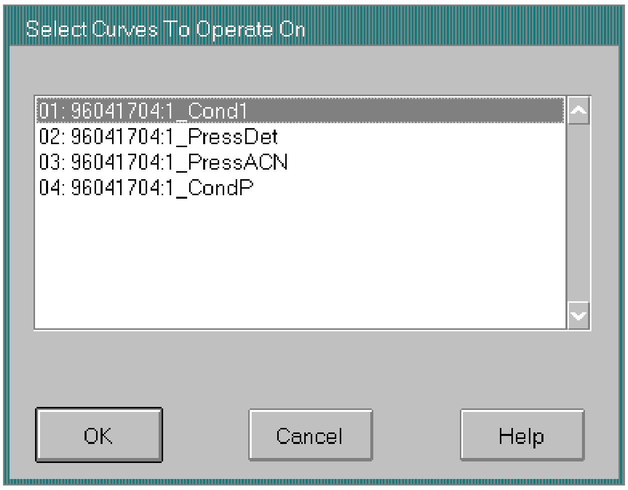
Another way to display only part of a curve is to 'fix' the minimum and maximum values of the y and or x axes in the **Chromatogram Layout** box. See Section 7.2.7.

Cutting curves

The **Operations:Cut curve** function allows a region of the curve between two values on the x-axis to be cut and stored as a new curve. This is done in the following way:

1. Select **Operations:Cut curve**.

2. A dialogue box will open in which you must select the curve to be operated on. Click on **OK**.

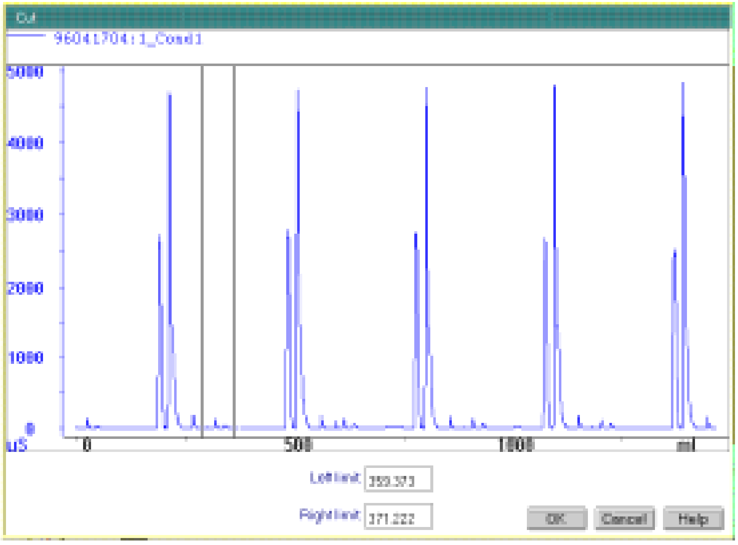


Select Curves To Operate On

01: 96041704:1_Cond1
02: 96041704:1_PressDet
03: 96041704:1_PressACN
04: 96041704:1_CondP

Figure 7-14. *Select Curves to Operate On dialogue box.*

3. The selected curve will now be shown in a new window which also contains two vertical cursor lines. To facilitate the cutting process, it is possible to use the zoom function within the window.



Left limit: 359.373

Right limit: 371.222

__Figure 7-15.__ Defining the limits of the curve to be cut using the cursor lines

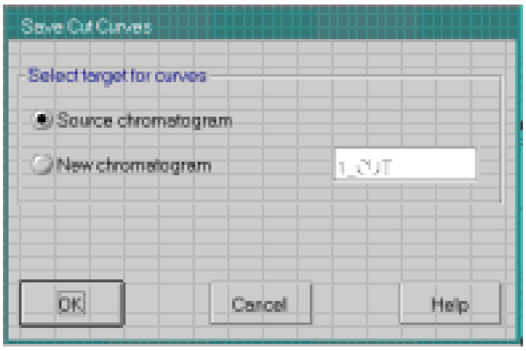
To make the cut:

- drag the two cursor lines to define the left and right limits of the cut area. *or,*

- type the desired left and right limit values in the boxes marked **Left limit** and **Right limit**.

Note: The areas outside of the **Left limit** and **Right limit** will not be saved in the newly created cut curve. Thus, the x-axis of the new saved curved will not begin at zero unless designated as one of the limits. The *original* curve is not changed by this operation.

4. Click on **OK**. A new panel will appear. Select whether to save the new cut curve in the **Source chromatogram** curve list, or in a **New chromatogram**. If you select the latter option, you can change the name of the new chromatogram. Click on **OK**.



Save Cut Curves

Select target for curves

● Source chromatogram

○ New chromatogram   1_CUT

Figure 7-16. *Save Cut Curve dialogue box.*

5. The original source chromatogram will be displayed. If the destination of the cut curve was the source chromatogram, open the **Chromatogram Layout** box and select the new cut curve from the curve list. If the destination of the cut curve was a new chromatogram, this will be represented as an icon. Double click on this icon to open it.

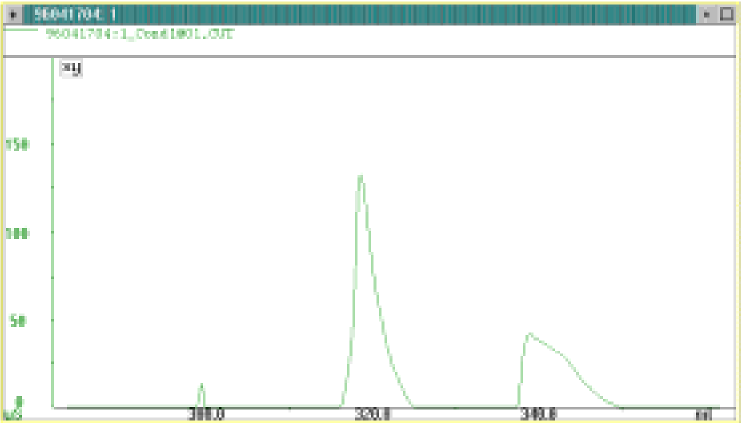


Figure 7-17. *Cut curve displayed in the chromatogram. The y-axis has been fixed to best display the curve (see Section 7.2.7).*

7.3.2 Entering text in the chromatogram

Up to five basic annotations can be added to the chromatogram.

1. Select **Edit:Text:Add**. The mouse pointer is replaced with an **ABC** pointer.

2. Position the pointer where you want to insert text in the chromatogram and click the left mouse button once.

3. In the dialogue box that appears, type the desired text and then click on **OK**. Now the text can be viewed on the chromatogram. The text is saved at the position where it is placed in the chromatogram window and is not linked to any curve. Text cannot be moved in the window once it has been placed.

If you want to edit the inserted text:

1. Select **Edit:Text:Edit**.

2. Select the specific text that you want to edit and make the appropriate changes and click on **Replace**. Click on **OK**.

If you want to delete inserted text:

1. Select **Edit:Text:Delete**.

2. Select the specific text that you want to delete and click on **Delete** and then **OK**.

7.4 Comparing different runs

The previous sections dealt with the manipulation of single curves within a chromatogram. The following sections describe how to make comparisons between two or more curves or chromatograms from different runs and details how best to present them.

It is possible to:

••view several chromatograms at the same time
••overlay curves from different runs in one chromatogram
••stack curves from different runs in one chromatogram
••stretch curves to make comparisons easier
••create mirror images

7.4.1 Comparing chromatograms from different runs

Two functions can be used to import chromatograms from other result files into an already opened result file, namely **File:Open related** or **File:Open**. The former option is most useful in searching for many chromatograms in a specific directory based on defined selection criteria. The latter option is best used to import any individual chromatograms from result files in different directories. The imported chromatograms will be sequentially numbered for identification purposes. Up to 10 chromatograms can be made available at the same time on the evaluation workspace.

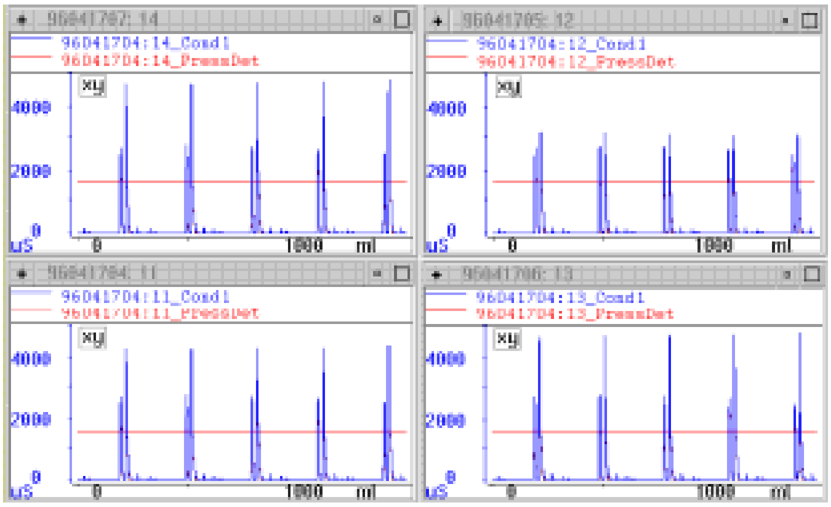
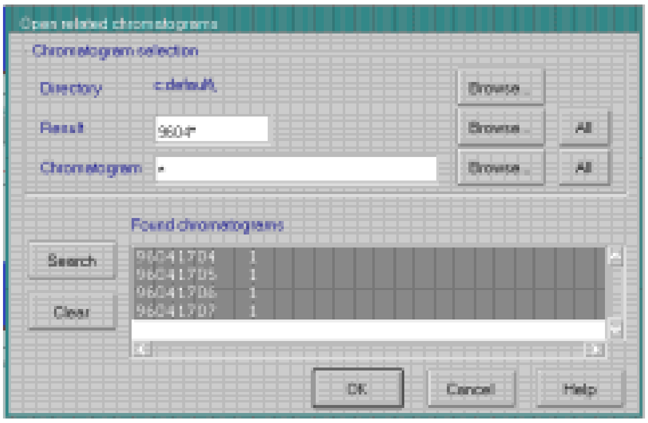


Figure 7-18. *Windows:Tile function to display many chromatograms. To print a picture like this, click on the Evaluation title bar at the top of the window and then press the <PrintScreen> key on the keyboard.*

Alternative A: Import chromatograms using Open Related

1. Click on **File:Open related** and select **Chromatograms**.



Directory c:default\

Result 9604*

Chromatogram *

```
96041704    1
96041705    1
96041706    1
96041707    1
```

Figure 7-19. *File:Open related chromatograms dialogue box.*

2. Select the directory and/or result file to be searched using the **Browse** options. A single asterisk(*) indicates that **All** has been selected. Note that you cannot search result files within sub-directories of the directory you have chosen. You must instead select the specific sub-directory to be searched.

3. You may instead want to search for result files and/or chromatograms with a specific name type, e.g. all result files with the name **9604** and all chromatograms contained therein. In this example, by typing **9604*** in the **Results** field and clicking on **All** for the chromatograms, the chromatograms for all result files with the same first four characters will be located in the search within the selected directory.

   You can use standard OS/2 wildcard characters in the file name specification (* represents a string of alphanumeric characters and ? for any single character). For example:

9604	will display only files named 9604
9604*	will display all files with names beginning 9604
***9604**	will display all files with names ending 9604
?9604	will display only 5-character names ending in 9604

4. Click on the **Search** command button and a list of chromatograms will be displayed based on the designated search criteria. A new search can be performed with new search criteria without erasing the first found chromatograms from the list.

5. If you do not want to include a chromatogram in your list, deselect it. Click on **OK** and all selected chromatograms are transferred to the Evaluation workspace and displayed as icons.

Alternative B: Importing using Open

1. Click on **File:Open** and then select **Chromatogram**.

2. Select the desired result file by double clicking on it, and all of the chromatograms contained within will be displayed.

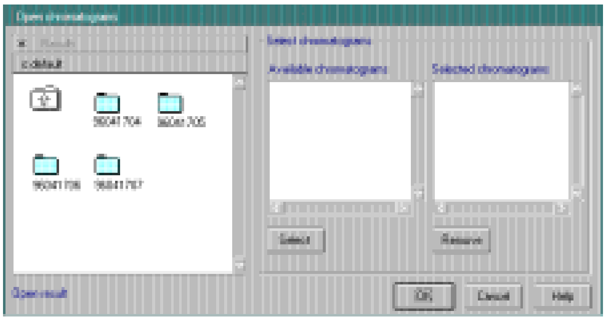


Figure 7-20. *File:Open chromatogram dialogue box.*

3. Select in turn the chromatogram(s) of interest and press the **Select** command button for each chromatogram. Selected chromatograms will be added to the **Selected Chromatograms** field. Chromatograms can be deselected by using the **Remove** command button.

4. Repeat steps 2-3 for chromatograms in other result files.

5. Click on **OK**.

Viewing all chromatograms

1. Double click on the imported chromatogram icons to open them.

2. Simultaneously display the chromatograms by selecting **Windows:Tile** or stack them by selecting **Windows:Cascade**.

3 a) Chromatogram windows can be individually sized and the presentation of the curves changed.

b) If you want to have the same scale on all of the chromatograms, open the **Chromatogram Layout** box for any chromatogram, make the changes and select (check) the **Apply to all chromatograms** option

4. To print all chromatograms on one page as they appear on the screen, click on the **Evaluation** title bar at the top of the window and then press the <PrintScreen> key on the keyboard.

7.4.2 Overlaying curves

Curves from different runs can be overlaid in one chromatogram. Curves can be copied into one chromatogram either from other chromatograms that are present on the Evaluation workspace, or be imported from other result files using **File:Open related** or **File:Open**.

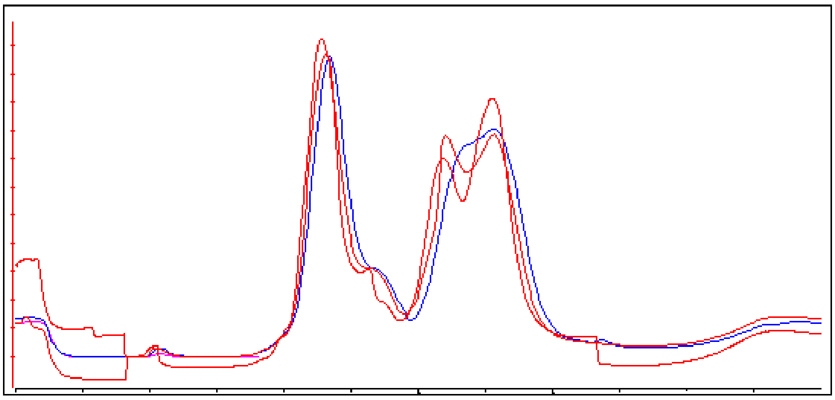


Figure 7-21. *Comparison of different curves.*

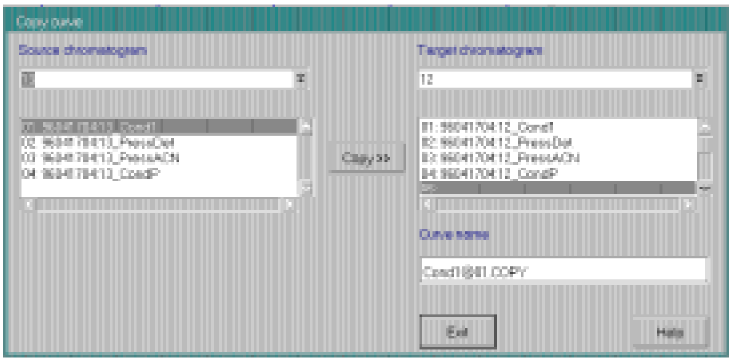
Alternative A: Copying curves into one chromatogram

For effective comparison of curves, it is suitable to transfer all relevant curves to a single chromatogram. This can be achieved by creating a new chromatogram, using **File:New:Chromatogram**, and copying curves into it from other chromatograms present on the Evaluation workspace. Alternatively, you can copy an existing chromatogram using **Edit:Copy:Chromatogram** and import more curves into it, or else copy curves into the **Temporary** chromatogram (see Section 7.1.3). You can create the desired curves, perform evaluations in the **Temporary** chromatograms and transfer the final curves to other destination chromatograms. The unwanted contents remaining in the **Temporary** chromatogram can then be removed using **Edit:Clear temporary chromatogram**.

1. Select **Edit:Copy:Curve**.

2. Select the source chromatogram and the curve of interest. Select the target chromatogram. Click on the **Copy** command button to effect the copy. Stay within the same dialogue box to repeat this step for as many other curves you want, from the same or different chromatograms. When you have copied all desired curves, click on **Exit**.

3. Open the target chromatogram and its **Chromatogram Layout** box. Select the curves that you want to view. Curves can be scaled individually or all with the same scale using the **All with this unit** function (see Section 7.2.7).



Target chromatogram: 12

01 960417B413_Cond1
02 960417B413_PressDet
03 960417B413_PressACN
04 960417B413_CondP

01: 960417Q412_Cond1
02: 960417Q412_PressDet
03: 960417Q412_PressACN
04: 960417Q412_CondP

Curve name: Cond1@01.COPY

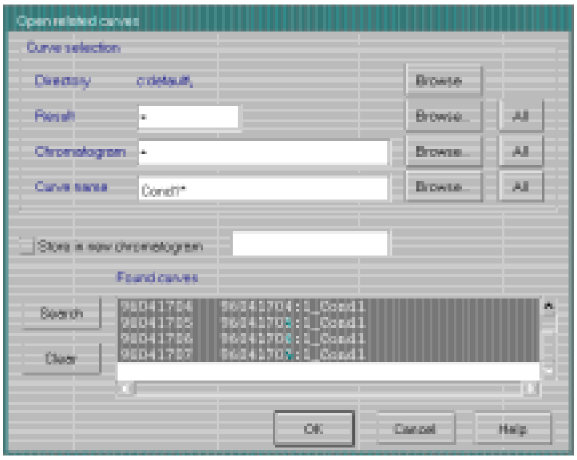
Figure 7-22. *Edit:Copy curve dialogue box.*

Alternative B: Importing curves using Open related

Result files contained in the same directory can be automatically searched to locate all curves of a specified type, for example, all Cond1 curves.

1. Select **File:Open related:Curves**. Select the search criteria for the directory, result chromatogram and curve name using the respective **Browse** command buttons. Alternatively, click on the respective **All** button to include all result files, chromatograms and/or curves.

   Wildcard characters, * and ?, can also be used to specify the search parameters (see Section 7.4.1 under *Alternative A*).



Directory c:default\

Result *

Chromatogram *

Curve name Cond1*

```
98041704    98041704:1_Cond1
98041705    98041705:1_Cond1
98041706    98041706:1_Cond1
98041707    98041707:1_Cond1
```

Figure 7-23. *File:Open related curves dialogue box.*

2. Click on **Search** and a list of found curves will be displayed based on the designated search criteria. A new search can be performed with new search criteria without erasing the chromatograms located in the previous search.

3. Deselect the curves you do not want to be imported.

4. If wanted, select (check) the **Store in new chromatogram** option. Enter a name for the new chromatogram.

   This option is recommended, since all searched curves can be kept separate from the original chromatogram.

5. When you have made your selection, click on **OK**. If you selected for a new destination chromatogram to be created, its icon will now appear in the workspace. Otherwise, the chosen curve(s) will instead have been imported into the active chromatogram.

6. Double click on the icon for the target chromatogram and open the **Chromatogram Layout** box. Select the curves that you want to view. Curves can be scaled individually or all with the same scale using the **All with this unit** function (see Section 7.2.7).

Alternative C: Importing curves using Open
Using the **File:Open:Curves** function, individual curves may be imported into the active chromatogram.

1. Ensure that the destination chromatogram for the imported curve(s) is active on the screen.

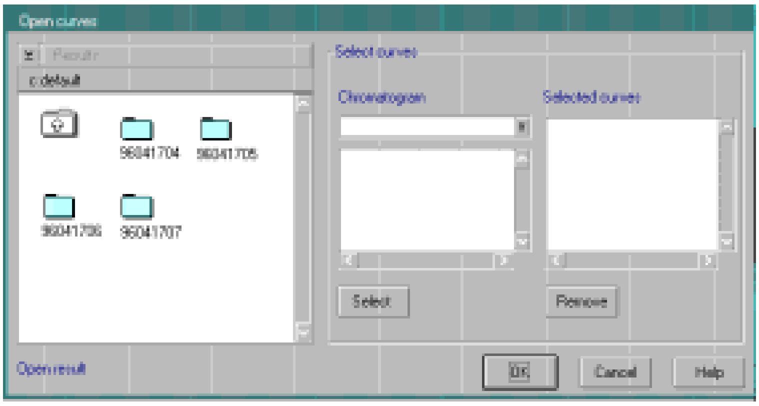


Figure 7-24. *Open curves dialogue box.*

2. Select **File:Open:Curves**. A dialogue box will appear containing all results files.

3. Click on the result file of choice and, where appropriate, the specific chromatogram containing the desired curve. The chosen chromatogram and curves contained therein will be listed in the **Select curves** field.

4. Select the desired curve and click on the **Select** command button. The selected curve will now be displayed in the **Selected curves** field.

5. If you want to choose curves from the same or other chromatograms, repeat steps 3-4 for each curve. When all the desired curves have been selected, click on **OK**.

6. Double click on the icon for the new chromatogram and access the **Chromatogram Layout** box. Select the curves that you want to view. Curves can be scaled individually or all with the same scale using the **All with this unit** function (see Section 7.2.7).

7.4.3 Stacking and stretching curves

When two or more curves from different runs are displayed on the same chromatogram, it is often difficult to visualize the differences between them due to the high degree of overlapping. This makes it difficult to align the curves and to make comparisons between individual features. Several tools are available to overcome such problems, namely normalizing curves, shifting curves and stretching curves. The operations presented below require the curves to be present in one chromatogram (see Section 7.4.2).

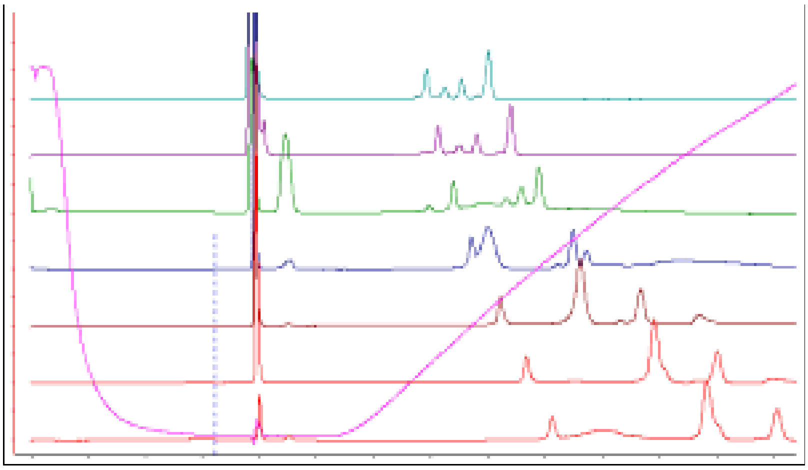


Figure 7-25. *Stacking curves.*

Alternative A: Stacking and stretching curves using the normalize function

The simplest method to align curves with respect to the x-axis or the y-axis, is to use **Operations:Normalize**.

To select the curve to be moved within a chromatogram:

1. Select **Operations:Normalize**. A panel will appear.

2. Select the curve to be *normalized* and a reference curve to be *normalized against*. For example, if you want to stack curves, select the curve at the bottom of the stack to be *normalized against* and the curve to be moved as *normalized*. Click on **OK**.

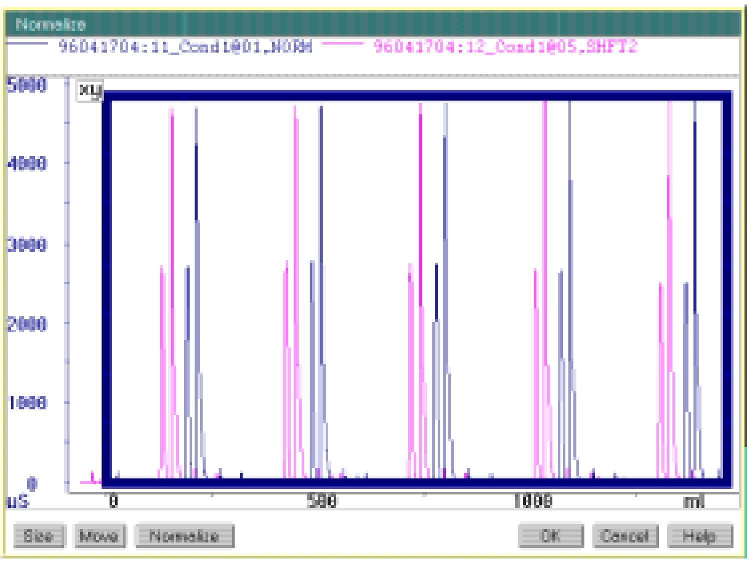


Figure 7-26. *Normalize chromatogram window.*

3. The **Normalize** window is displayed. A box surrounds the curve selected to be normalized. You can now use the following functions:

Move Allows the selected curve to be moved to any position on the chromatogram. Axes are automatically re-scaled to accommodate the new position. This function is useful for stacking curves. Click on **Move** and then move the curve with the mouse pointer. Click on the mouse button when the curve is in the correct position.

Size Allows the selected curve to be stretched along its y-axis or x-axis. Click on **Size** and then drag the box along either one of its axes. This is useful, for example, to stretch a curve along its y-axis for comparison of different gradient lengths.

Normalize The curve to be normalized will be adjusted to the reference curve. Thus, the height of the highest peak on both curves will be the same and will occur at the same retention point. The curve to be normalized is automatically moved along the x-axis and stretched along the y-axis.

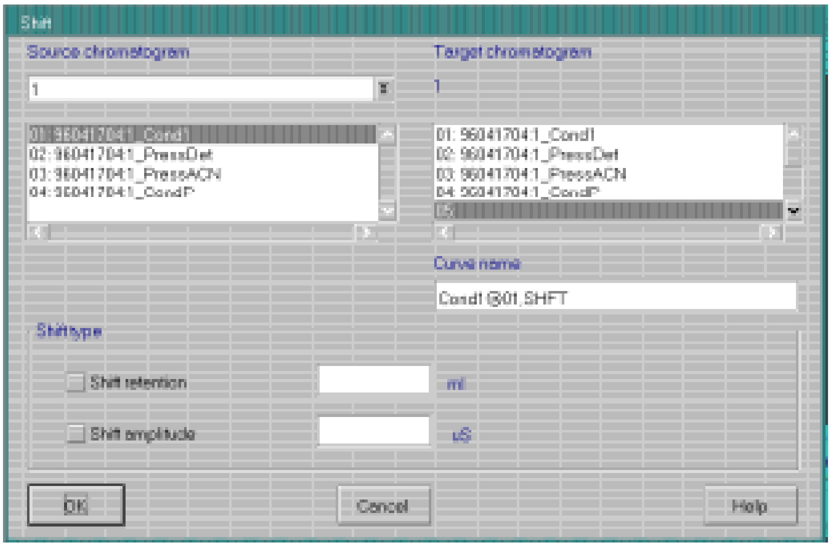
4. When all operations have been performed, click on **OK** to save the new normalized curve. Open the **Chromatogram Layout** box to select the curves you want to see.

5. Repeat the procedure for other curves you want to stack or stretch.

Alternative B: Moving a curve using the Shift function
If more precise positioning of curves is required, then **Operations:Shift** should be used. Use of this function is logged in the evaluation log.

1. Select **Operations:Shift** and then the curve to be shifted in the displayed dialogue box.

2. Select the axis along which the shift will be made, i.e. along the x-axis (**Shift retention**) or y-axis (**Shift amplitude**). Enter the shift value. Click on **OK**.



Source chromatogram: 1

01: 960417041_Cond1
02: 960417041_PressDet
03: 960417041_PressACN
04: 960417041_CondP

Target chromatogram: 1

01: 960417041_Cond1
02: 960417041_PressDet
03: 960417041_PressACN
04: 960417041_CondP
05:

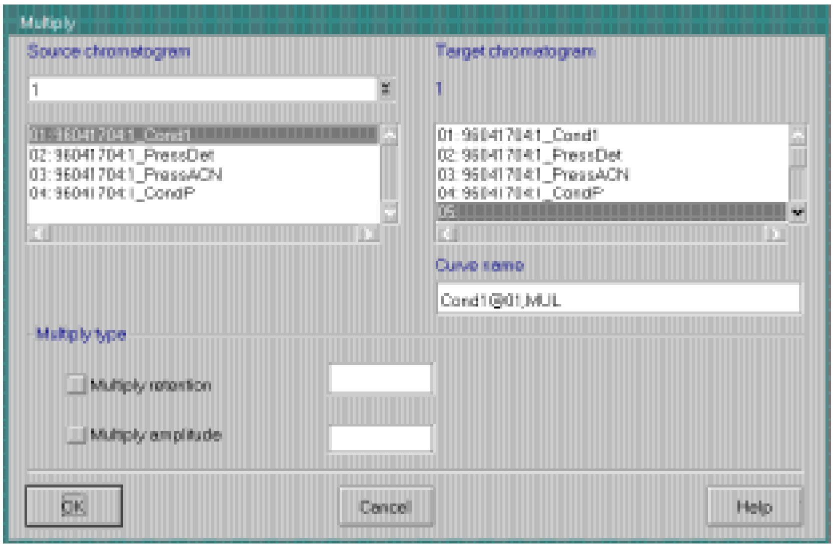
Curve name: Cond1@01,SHFT

□ Shift retention

□ Shift amplitude

Figure 7-27. *Shift dialogue box.*

Alternative C: Stretching and shrinking a curve using Multiply
Curves can be stretched or shrunk in the x or y plane using the **Multiply** function. This function is similar to **Normalize:Size** except that each curve is repositioned with precise numbers instead of by eye, and the is instruction logged in the evaluation log. Click on **Operations: Multiply** and select the curve to be multiplied. Select (check) the appropriate axis for multiplication, either **Multiply retention** and/or **Multiply amplitude**. Insert the appropriate multiplication factor and click on **OK**.



Source chromatogram: 1

01:9604170411_Cond1
02:9604170411_PressDet
03:9604170411_PressACN
04:9604170411_CondP

Target chromatogram: 1

01:9604170411_Cond1
02:9604170411_PressDet
03:9604170411_PressACN
04:9604170411_CondP
05:

Curve name: Cond1@01,MUL

Multiply type

□ Multiply retention

□ Multiply amplitude

Figure 7-28. Multiply dialogue box

7.4.4 Mirror images of curves

A very useful way of comparing the features of two curves is to produce a mirror image of one curve. To achieve this:

1. Select **Operations:Multiply**.
2. Select the desired curve to be *mirrored* and select **Multiply amplitude** in the **Multiply type** field.
3. Type in a multiplication integer of -1 and click on **OK**.
4. Shift the mirror image curve downwards for better presentation (See *Alternative B*, above)

   Now the mirror image of the original curve will be displayed in the active window. Select/deselect for the other curves wanted in the active chromatogram window in the **Chromatogram Layout** box.

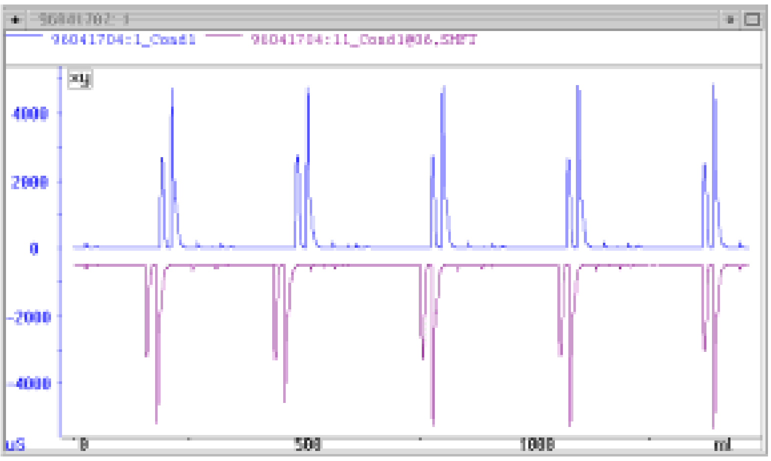


Figure 7-29. *Two curves displayed in mirror image.*

7.5 Saving results

Any changes to chromatograms, including all new created curves and all imported or created chromatograms, can be saved in two ways; either using **File:Save**, which saves all changes in the original result file, or using **File:Save as**, which allows you to create a copy of the result file under a new name.

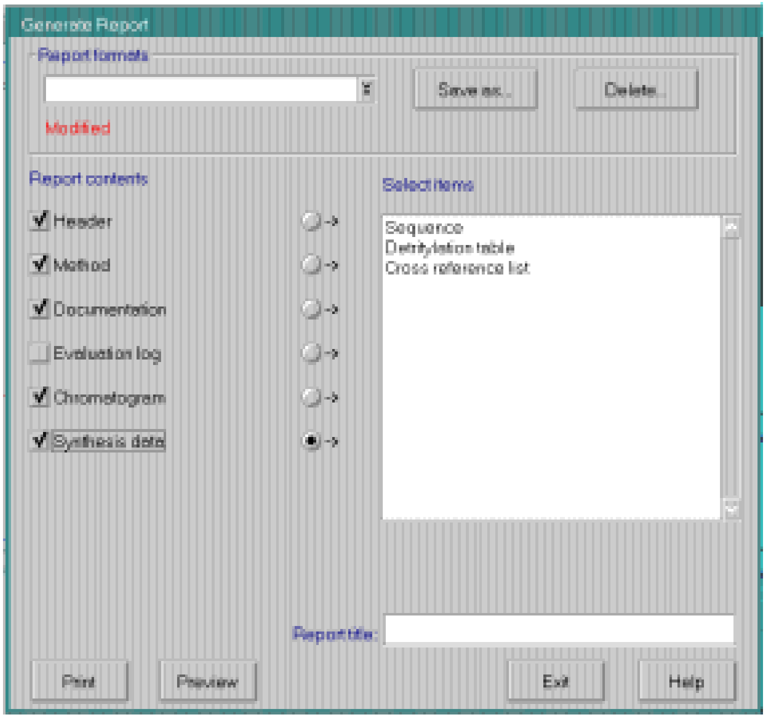
Note: All curves created during the manipulations will also be saved. This may not always be desirable. Before saving, remove unwanted curves from a chromatogram using **Edit:Delete:Curve**. The original curves can never be deleted.

7.6 Printing chromatogram and documentation reports

A printed record of all documentation concerning a run, including the method and Run Log, can be obtained as follows:

1. Select **File:Report** and a dialogue box will appear. If you have predefined report formats, you can choose one and then press **Print**.

2. Select the main items to be included in your report by placing a check mark in the box beside each of the relevant options under **Report contents**.



☑ Header ○
☑ Method ○
☑ Documentation ○
□ Evaluation log ○
☑ Chromatogram ○
☑ Synthesis data ●

Sequence
Detritylation table
Cross reference list

Figure 7-30. Synthesis data options in the Generate Report box.

3. Associated with each report content item is a radio button. Selection of a radio button lists a subset of options linked to the specific report contents option. Select the options which are to included in the report.

Note: It is possible to press a radio button and select items even if the corresponding report contents option is not selected. Information will only be included in the report if the appropriate option under **Report Contents** is selected.

4. When you have decided on the report contents, you can preview the report by clicking on **Preview** and/or print the report by clicking on **Print.**

Two of the report options warrant more detailed description, namely **Synthesis data** and **Chromatogram**.

Synthesis data

This option can be used to print out all of relevant information concerning the actual synthesis, including the sequence, detritylation table and cross-reference list. The detritylation table contains information about the efficiency of the coupling reaction for the addition of each base to the oligonucleotide. Efficiency is automatically calculated by measuring the conductivity in the cell during the cleavage of DMTr at the detritylation step. The level of conductivity is determined by the amount of cleavage and is directly correlated to the area under the relevant peak in a coupling cycle. The area is determined by peak integration, which is automatically performed if one of the supplied method templates has been used to create a method. Click on the appropriate radio button and select the options to be included.

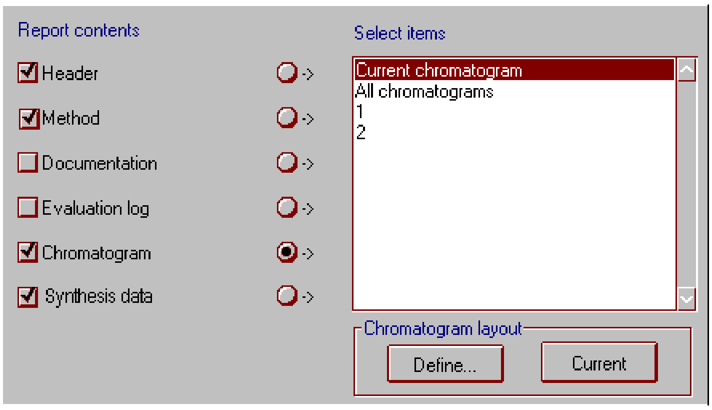
Chromatogram

Selection of the radio button associated with the report content, **Chromatogram**, allows you to specify which chromatograms and/or chromatogram components that are to be included in the report.

1. Click on the radio button for **Chromatogram**.

2. Make a selection from the item list:

Current chromatogram	The chromatogram that is currently active on the screen
All chromatograms	All chromatograms on the evaluation desktop, including those that are shown as icons
1,2,...etc.	An individually named chromatogram



Report contents

☑ Header ○ ->

☑ Method ○ ->

□ Documentation ○ ->

□ Evaluation log ○ ->

☑ Chromatogram ● ->

☑ Synthesis data ○ ->

Select items

Current chromatogram
All chromatograms
1
2

Figure 7-31. *Selection of chromatogram content in the Generate Report dialogue box.*

3. If you want the chromatogram(s) to be printed out as they currently appear on the screen, click on the **Current** button. The layout will be the present layout for each chromatogram on the evaluation desktop.

If you want the chromatogram(s) to be printed out with a different layout as they currently appear on the screen, click on the **Define** button. This opens the **Chromatogram Layout** box and enables changes to be made to the layout. Note that the changes made here within the **Chromatogram Layout** box are only with respect to the report format and not the layout of the chromatograms displayed on the screen.

Various report layout changes can be made in the **Chromatogram Layout** dialogue box. For example:

- curves
- axes
- header information to be included on the same page as the chromatogram
  Select **Header** in the **Chromatogram Layout** box. Select Variables and/or Questions. Header information for imported chromatograms can not be printed.

4. Click on **OK** when all changes in the **Chromatogram Layout** box have been performed.

7. Click on **Print** or **Preview**.

7.6.1 Saving a report format

you can save a customized report format for later use. A saved report format also includes any selections you made in the **Chromatogram Layout** box with respect to the report.

Note: The format cannot be saved with **Current** layout. The layout saved is always the one shown in **Define**.

All saved formats can be globally saved, i.e. made available for any user (if you have **Edit global lists** authorization), or can be specific for the current user only.

1. Select all the relevant information for your report format.

2. Click on the **Save as** command button and type in a name for your format in the box that appears. Click on **OK** to save the format.

Note: A format saved with the name DEFAULT (in upper-case letters) will be used as the default format for all result files when they are opened for the first time.

7.6.2 Selecting a format

The newly saved format can be applied to any new report by selecting it from the list of **Report Formats**.

Note: A report format saved with the **Current chromatogram** does not necessarily print the chromatogram as it appears on the screen. The layout (curve, axis etc.) that will be printed is the one which is found under **Define**. If you want to print the chromatogram as it appears on the screen, click on the **Current** (layout) button and then on **Print**.

Some global report formats are provided with the installation. Do not delete these formats, since you will then be unable to run the corresponding procedures.

If you want to print a number of results with the same report format, create a procedure to print one result and then perform a batch run for the required results (see Section 8.3.6).

7.6.3 Printer set-up

To change your choice of destination printer for Evaluation printouts, select **File:Printer setup** and select the desired printer. Within the **Printer setup** dialogue box, you can change the job properties by clicking on the appropriate command button. A new box will open which allows you to choose from the standard printer options, for example, paper size, portrait or landscape printing, form feed control. Print set-ups are saved on clicking **OK**. Note that printing from other parts within UNICORN OS is performed via the default OS/2 printer.

7.6.4 Printing active chromatograms

To print out the active chromatogram, select **File:Print**. If the Documentation is open, **File:Print** allows you to print any components in the documentation.

7.7 Exiting Evaluation

If you want to quit from the Evaluation module of UNICORN OS, select **File:Exit**. You will then be asked if you want to save the results of the evaluation session that you have performed. If you answer **Yes**, the previous version of that result file will be unconditionally overwritten. This may be undesirable if you have included the current result file within an evaluation procedure batch run (see Section 8.3.5).

8. Evaluating results

This chapter will mainly describe how to:

- integrate peaks
- automate evaluation operations
- export data and curves

8.1 Integrating peaks

UNICORN OS allows you to identify and measure on a curve such values as peak areas, retention times and peak widths. This uses peak integration and should be used if you are not satisfied with the automatic integration performed during a run, or if the automatic integration function was not used.

8.1.1 Baseline calculation for integration

Integrating peaks is divided into two steps: calculating the baseline and calculating peak areas. As a correct baseline is crucial for accurate calculation of peak areas, several ways of calculating the baseline are available in UNICORN OS:

- Using the **Calculate baseline** instruction for automatic calculation of the baseline, which gives, in most cases, a very accurate measurement. This is the most common alternative and it is recommended that you read the information contained within Appendix C.2 which describes the principles of baseline creation.

- A blank run with the same chromatographic conditions as the sample can be used as the baseline for peak integration.

- In addition to blank runs, it is possible to select any curve present in the current chromatogram, e.g. an edited baseline (see Section 8.1.4), as baseline.

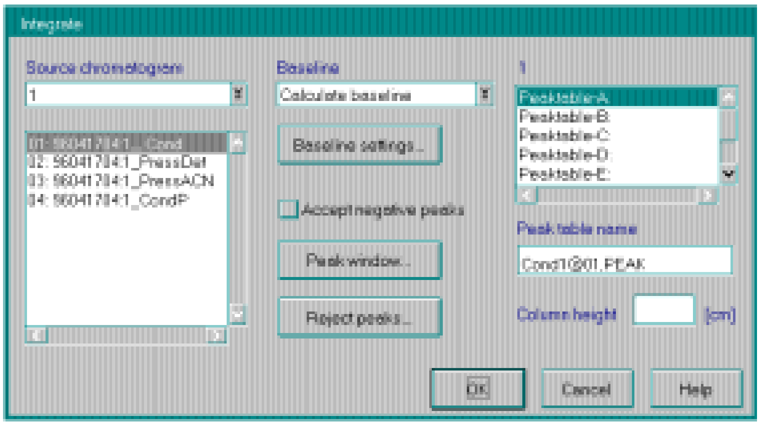
- Using a **Zero baseline**, i.e. no baseline subtraction at all.

- If a baseline for the selected source curve already has been calculated using **Integrate:Calculate baseline**, it can be reused by selecting the **Correlated baseline** option. This is the default alternative whenever possible.

8.1.2 Performing a basic integration

To perform a basic integration

1. Select **Integrate:Peak integrate**. The **Integrate** box will open.



Integrate

Source chromatogram: 1

01: 960417841_Cond
02: 960417841_PressDet
03: 960417841_PressACN
04: 960417841_CondP

Baseline: Calculate baseline

□ Accept negative peaks

1: PeaktableA, PeaktableB, PeaktableC, PeaktableD, PeaktableE

Peak table name: Cond1@01,PEAK

Column height: [cm]

Figure 8-1. *Integrate dialogue box.*

2. Choose the source curve to be analyzed, usually the Cond1 curve, and a peak table destination in the peak table list. Any one chromatogram can contain up to eight peak tables, designated A-H.

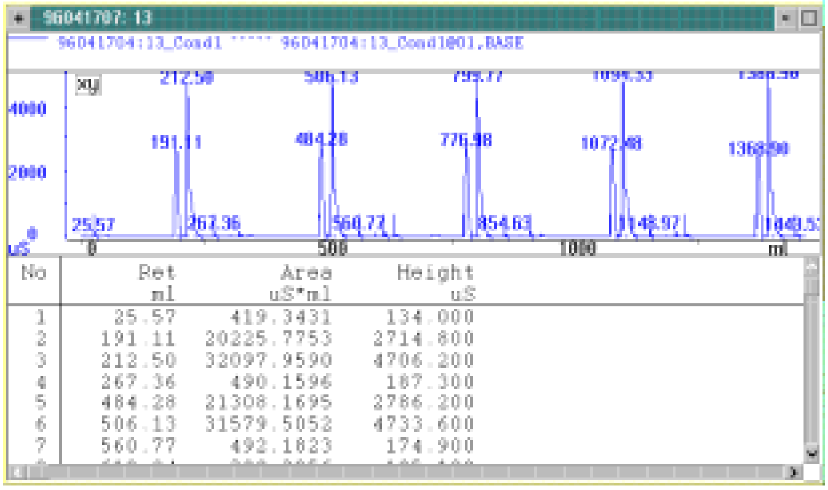
3. Make the appropriate **Baseline** selection from the above-mentioned possibilities.

   The **Calculate baseline** option with the default settings for the parameters (displayed if the **Baseline settings** command button is pressed) is the most common choice.

4. Click on **OK** to perform the peak integration when you are satisfied with your selection.

Following integration, the peaks in the chromatogram will be automatically labelled with their respective retention. The start and end point of each peak will be marked by drop-lines.

The peak table will be displayed underneath the active chromatogram.



No	Ret ml	Area uS*ml	Height uS
1	25.57	419.3431	134.000
2	191.11	20225.7753	2714.800
3	212.50	32097.9590	4706.200
4	267.36	490.1596	187.300
5	484.28	21308.1695	2786.200
6	506.13	31579.5052	4733.600
7	560.77	492.1823	174.900

__Figure 8-2.__ The results after peak integration.

In addition to peak areas, several other peak characteristics such as retention time and peak width are automatically calculated. The characteristics displayed in the peak table may be selected in the **Chromatogram Layout** box (see Appendix C.3). All characteristics are automatically calculated for each integrated peak when the **Peak Integrate** function is used, although only the selected items will be displayed in the peak table.

Changing peak labels
As an alternative to using retention as the peak label, the peaks can be sequentially numbered or be marked with specific identification tags that can be set in the **Integrate:Edit Peak table** function. The choice of label type is made via the **Tag** command button in the **Chromatogram Layout** box. Note that the labels may be displayed vertically for each peak by deselecting the **Horizontal text** option.

Filtering peaks from view
It is possible to temporarily remove peaks from view in a peak table based upon the criteria you determine. In the **Chromatogram Layout** box, click on the **Filter peaks** command button in the **Peak tables** field. A box will appear which is similar to the **Reject peaks** function in the **Peak Integrate** function dialogue box. Select (check) the criteria and parameters by which peaks will be excluded from your peak table view. You are able to define the minimum height, width and area, the maximum width as well as a specified number of the largest size peaks. Click on **OK**.

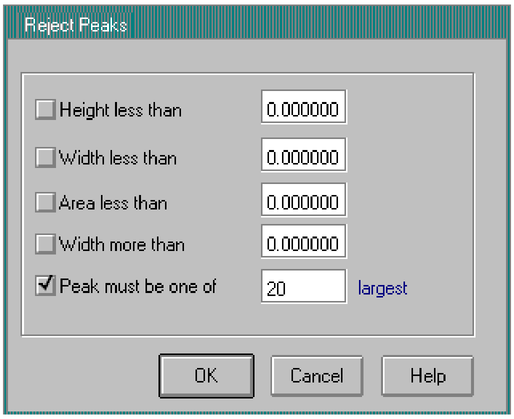
If you later want to include the peaks again you have to deselect the criteria in the **Filter peaks** dialogue. The difference between **Filter peaks** and **Reject peaks** is that the latter function permanently excludes peaks from the integration and affects the calculation of total peak area etc.

8.1.3 Optimizing peak integration

If the results from the peak integration are unsatisfactory, there are several possibilities to improve the results.

1. Changing the parameters used in the **Calculate baseline** instruction can help to optimize the integration result. The baseline parameters can be altered in two possible locations (i) in the dialogue box which appears after selection of **Integrate: Calculate baseline**, or; (ii) by selection of the **Baseline settings** command button in the resulting dialogue box from **Integrate:Peak integrate**. These are the same function except that in the latter case, the baseline is immediately used in an peak integration.

2. It is possible to exclude peaks from integration based upon criteria you determine. Click on the **Reject peaks** command button in the **Integrate:Peak integrate** dialogue. In the box that appears, select (check) the criteria and parameters by which peaks will be excluded from the integration. You are able to define the minimum height, width and area, the maximum width as well as a specified number of the largest size peaks. The default criteria is to include only the 20 largest peaks.



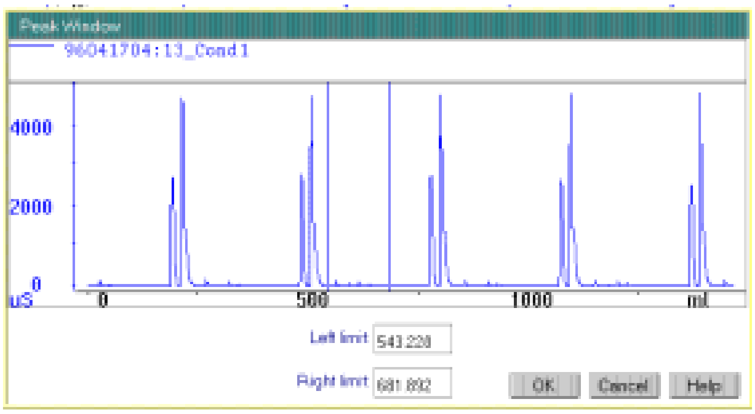
Reject Peaks

□ Height less than 0.000000
□ Width less than 0.000000
□ Area less than 0.000000
□ Width more than 0.000000
☑ Peak must be one of 20 largest

Figure 8-3. *Reject Peaks dialogue box.*

3. To select only a part of a curve for integration, click on the **Peak window** command button in the **Integrate:Peak integrate** dialogue box. A chromatogram will open containing the curve and two vertical cursor lines. These lines can be dragged to define a region between them that will be analyzed. Alternatively, x-axis values

for the **Left limit** and **Right limit** may be typed in. Click on **OK** to return to the main dialogue box. The baseline will be calculated from the whole curve, but calculation of the areas beneath the peaks is only performed on the selected section of the curve. The default peak window includes the entire curve.



Left limit 543.228

Right limit 681.892

__Figure 8-4.__ Peak Window chromatogram window.

4. If you want to include negative peaks in the integration, select (check) **Accept negative peaks** in the **Integrate:Peak integrate** dialogue box (or in **Integrate:Calculate baseline**). The negative peaks will be reported as negative areas in the peak table. By default, negative peaks are not included in the integration.

5. In the event that the automatic baseline calculation is not satisfactory, it is possible to edit the baseline manually by inserting and deleting baseline points. This is done with the **Integrate:Edit baseline** function (see Section 8.1.4).

6. The **Integrate:Edit peak table** function (see Section 8.1.5) allows you to manually adjust the peak start and end points, split and join peaks.

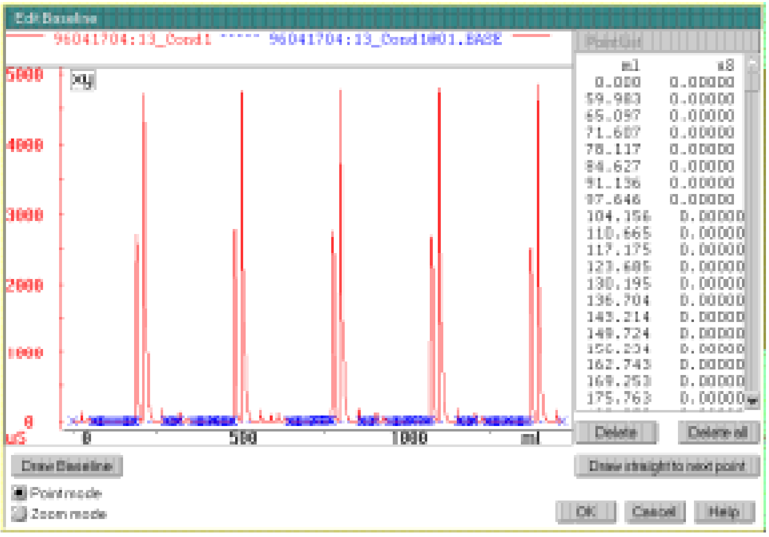
8.1.4 Manually editing a baseline

Once a baseline has been calculated, it is possible to add or remove baseline points on it and then draw a new baseline from the new set of data points. The edited baseline curve can then be used in a new peak integration.

1. Select **Integrate: Edit baseline**.

2. Choose the desired baseline from the panel that appears and click on **OK**. A window will appear displaying the baseline and the

curve from which it was calculated. Additionally, blue crosses are displayed (the baseline points) and their co-ordinates in the **Point list**.



ml	uS
0.000	0.00000
59.983	0.00000
65.097	0.00000
71.607	0.00000
78.117	0.00000
84.627	0.00000
91.136	0.00000
97.646	0.00000
104.156	0.00000
110.665	0.00000
117.175	0.00000
123.685	0.00000
130.195	0.00000
136.704	0.00000
143.214	0.00000
149.724	0.00000
156.234	0.00000
162.743	0.00000
169.253	0.00000
175.763	0.00000

● Point mode
○ Zoom mode

Figure 8-5. Edit Baseline chromatogram window.

Insertion of baseline data points
Select the **Point mode** radio button. Click on the left mouse button to place the point on the chromatogram. Each new point is represented by a cross and its co-ordinates are automatically entered into the **Point list**. This is useful when, for example, you want the baseline to go up to a high valley between two peaks. Use the right mouse button if you want to use the zoom function to magnify a region of the chromatogram. Selecting **Zoom mode** switches the mouse buttons for the two functions, i.e. the left mouse button controls the zoom function and the right mouse button is used to enter points.

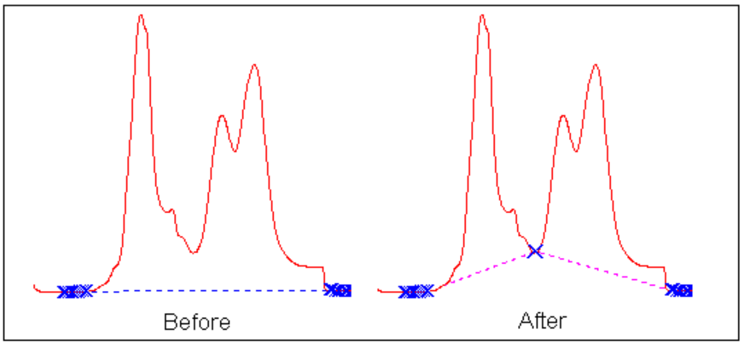


Figure 8-6. Baseline before and after editing.

Deleting baseline data points
If you want to delete a data point from the **Point List**, select the appropriate point in the **Point list** and press the **Delete** button. Alternatively, double click on the unwanted data point entry in the list to delete it. **Delete all** removes all baseline data points.

Drawing the new baseline
When you are satisfied with your baseline point selection, click on the **Draw Baseline** command button. The new baseline curve will be drawn as a spline function based on the previous and the new points. The spline function is guided by the points, but does not necessarily pass through them. You may also force a straight baseline between two points by selecting the first of the two points in the point list and then clicking on the **Draw straight to next point** command button.

Click on **OK**, and the new baseline will be saved with the default name **Edited Baseline**. This may now be used as the baseline in a new peak integration.

8.1.5 Adjusting the peak boundaries

Once a peak table has been generated using the appropriate baseline, it is now possible to split or join peaks and to manually adjust the peak start and end points. The peaks will then be renumbered and the peak areas will be recalculated.

It is suggested that you first open the **Chromatogram Layout** box, press the **Peak Label** command button and choose either **Number** or **Retention** for labelling the peaks. The former option will sequentially number each of the peaks in the chromatogram which is opened during the edit mode of a peak table. The latter option will display the retention value for each peak.

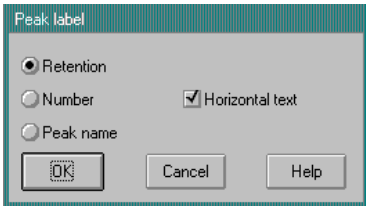
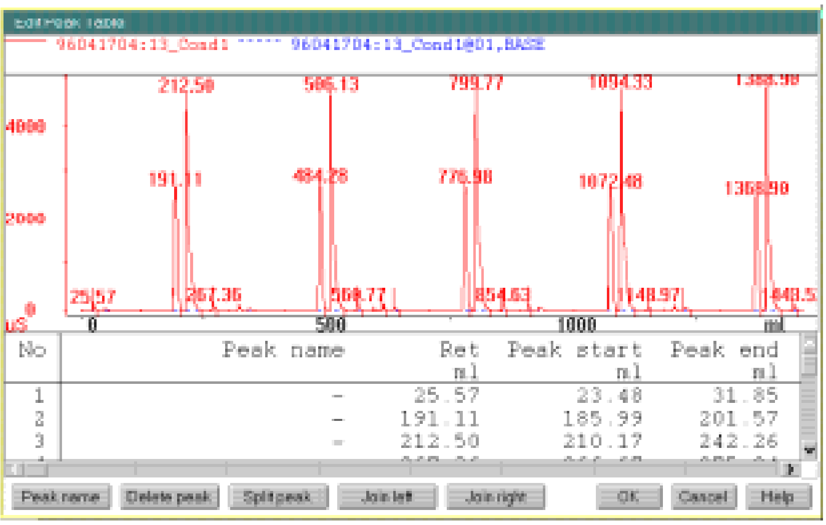


Figure 8-7. *Peak Label dialogue box.*

Select **Integrate:Edit peak table**. Choose the desired peak table from the dialogue box that appears. Note that name of the baseline on which the selected peak table was based, is displayed at the bottom of the dialogue box. Double click on the desired peak table in the list or click on **OK**. A chromatogram will appear containing the selected peak table with corresponding curve and baseline. The various editing features are described below. As an aid, it is possible to use the zoom function on the chromatogram in the **Edit Peak Table** window. Once you have completed your changes, click on **OK** and verify the destination of the new (edited) peak table.



No	Peak name	Ret ml	Peak start ml	Peak end ml
1	-	25.57	23.48	31.85
2	-	191.11	185.99	201.57
3	-	212.50	210.17	242.26

Figure 8-8. *Edit Peak Table chromatogram window.*

Deleting a peak in the peak table
To delete a peak from the table, click on the peak in the chromatogram or in the peak table and click on the **Delete Peak** command button. Note that the remaining peaks will be renumbered after the deletion.

Splitting a peak
A peak is defined within two limiting drop-lines; one to the left and right of the peak. It is possible to split the peak to create two new “peaks” by inserting a new drop-line. A new drop-line is always positioned at the middle point between the two existing drop-lines. Thus, the area of each new peak is dependent on the symmetry of the original peak.

To make a split, select the desired peak in the list or mark it in the curve. Press the **Split peak** command button.

Note: The peaks will be renumbered according to the split. Refer to the description below about adjustment of the drop-lines.

Joining a peak

It is possible to join the areas of adjacent peaks if separated by a drop-line.

1. Select a peak either on the chromatogram or in the peak table.

2. Click on **Join left** or **Join right** if you want the peak to be joined with the peak to its left or right respectively. The original intervening drop-line will now have been taken away and the peaks all renumbered.

Adjusting peak start and end points

The beginning of each peak is marked with a drop-line above the curve, and the end of each peak is marked with a drop-line below the curve.

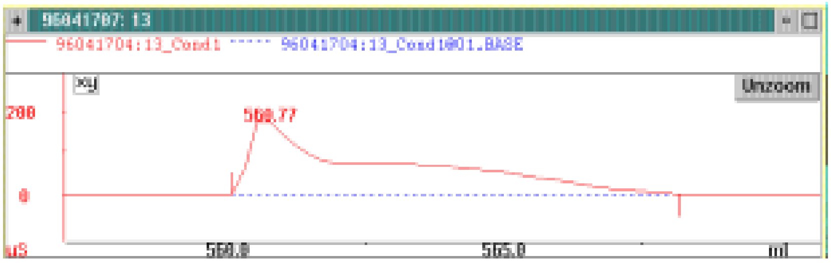


Figure 8-9. *A drop-line at the start and end of a peak.*

Where there are two peaks beside one another, the end of the first peak will be at the same point as the beginning of the next peak. Thus, there will be a drop-line below and above the line respectively at the same point.

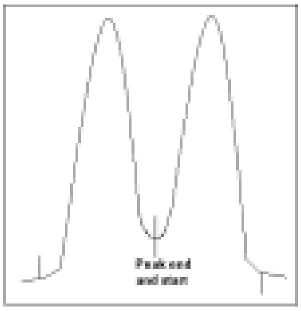


Figure 8-10. *A drop-line between two peaks.*

It is possible to move the drop-lines for a selected peak and thus affect the area beneath the peak.

1. Click on the peak of interest on the curve or in the peak table and two vertical cursor bars will become superimposed on the left and right drop-lines that delimit the selected peak.

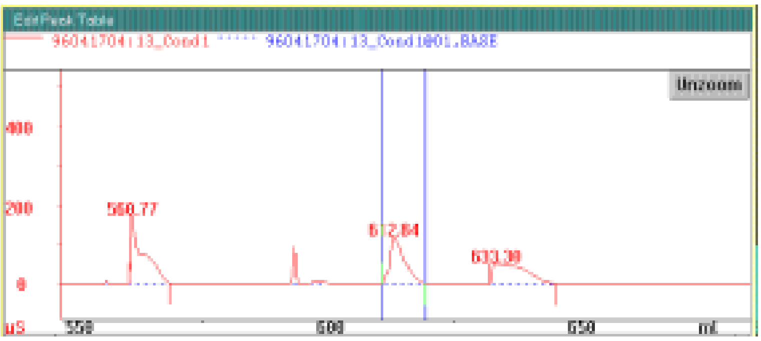


Figure 8-11. Active bars delimiting left and right drop-line for a peak.

2. Drag the left and right drop-line bars to define the new left and right limits respectively for the selected peak. The drop-lines can never be moved beyond any other drop-line. The new left and right boundaries are now represented by a drop-line above and below the curve respectively, and the peak areas are automatically recalculated.

3. Drop-lines on preceding or following peaks can be similarly adjusted. Movement of these drop-lines can be up to, but never beyond, any other drop-line. A drop-line may also not be moved beyond a point where the peak meets the baseline.

Identification names for peaks

Double click on the peak of interest in the list. A dialogue box will appear with the peak number, its retention value and a text field in which you can enter a specific tag for that peak. Click on **OK**. You will see the peak names in the chromatogram only if you have earlier accessed the **Chromatogram Layout** box, pressed the **Peak Label** command button and chosen **Peak names** for labelling the peaks.

8.1.6 Measuring HETP

HETP (height equivalent to a theoretical plate) calculations allow you to check how well the column has been packed.

1. Perform a run with injection of a non-interactive substance, for example, a small volume of acetone. Note that the injection must be at zero time.

2. In the **Integrate** dialogue box, type in the column height (cm) in the appropriate field area. Perform the peak integration according to your other selected parameters.

3. View the results of the integration, and select (highlight) **Plate height (HETP)** in the **Peak Table Columns** list of the **Chromatogram Layout** box.

Every peak will have a HETP value. A narrow peak gives a low value corresponding to a well packed column. A broad peak gives a high value, indicating a column that is not optimally packed.

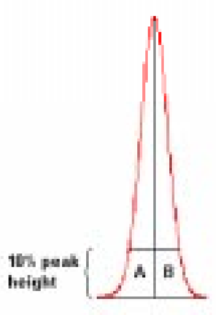
HETP is calculated as follows:

$$HETP = L/N$$
$$N = 5.54 * (V_R/w_h)^2$$

where

N = no. of theoretical plates
L = bed height in cm
V_R = peak elution volume or time
w_h = peak width at half height expressed in the same units as V_R

8.1.7 Measuring peak asymmetry



This function can be used in combination with HETP to help assess column performance. A perfect peak will have no asymmetry and, after peak integration, give a value of 1.0. Any value less than 1.0 means that there is a left skew, i.e. the asymmetry falls on the leading side (left) of the peak. The reverse is true for values greater than 1.0 where the asymmetry comes on the tailing side (right) of the peak.

To view the asymmetry data, open the **Chromatogram Layout** box and select **asymmetry** from the list in the **Peak Table Columns** list. Click on **OK** and return to the chromatogram.

Asymmetry = width B / width A, where A and B are measured at 10% of the peak height.

8.2 Other evaluations

8.2.1 Finding the slope values for Watch instructions

It is possible to set up conditional (Watch) instructions which allow the progress of a run to be determined by the events during the run, e.g. start integration when the first peak emerges. Watch instructions may rely on the slope of the curve as a parameter by which to initiate a specific change in the run. It is therefore important to use accurate slope values for the specific Watch instruction parameter.

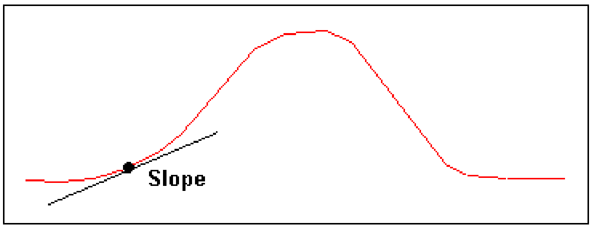


Figure 8-12. *The slope of a curve.*

To determine slope values, you must first make a run with the sample you intend to purify. Then use this result to find slope values in the Evaluation module:

1. Ensure that you have selected **Time** as the x-axis scale for retention in the **Chromatogram Layout** box.

2. Select **Operations:Differentiate**. Select the desired (Cond1) curve, check that a **First order** calculation is selected and click on **OK**. The differentiated curve will appear in the active chromatogram.

3. Measure the y-axis values on the differentiated curve by clicking on the **XY** icon, choosing the y-axis differentiate scale and reading the curve co-ordinates in the active **XY** box. It may be necessary to smooth the differentiated curve. The units for the differentiated curve is µS/min. Co-ordinates are based upon the position of the vertical cursor line on the chromatogram in relation to where it bisects the curve. Any y-axis value for the differentiated curve is the UV curve slope at the selected retention point.

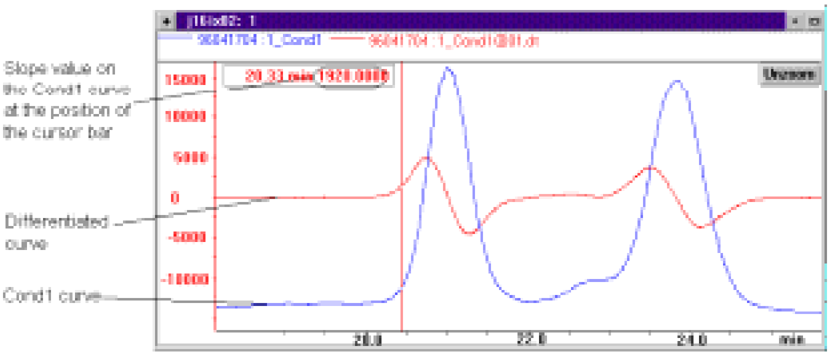


Figure 8-13. *Measurement of the Slope limit after differentiation.*

4. Use the zoom function to magnify the curve over an appropriate area. Place the vertical bar at the beginning of a peak where you want the Watch conditions to be fulfilled, i.e. where the slope becomes higher. Read the actual slope value in the active **XY** box.

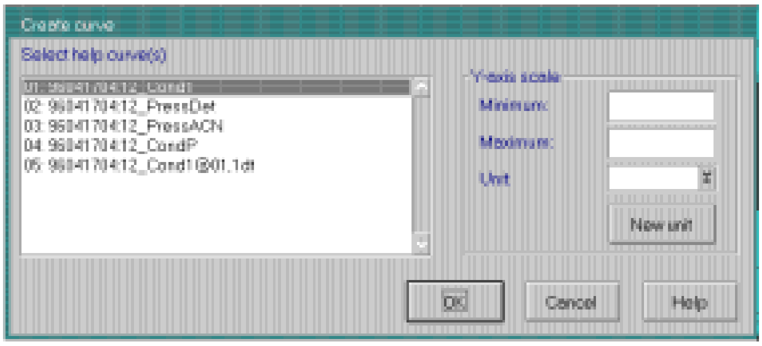
5. In the Method editor, enter the slope value as a parameter for the Watch instruction.

8.2.2 Creating a curve

It is possible to create a curve based on any external measurements.

1. Select **Operations:Create Curve**. In the box that is displayed, select a help curve.

2. Select the minimum and maximum values of the y-axis. Also choose the appropriate units from the list that is displayed when you click on the drop-down arrow. The help curve determines the min. and max. values for the x-axis.



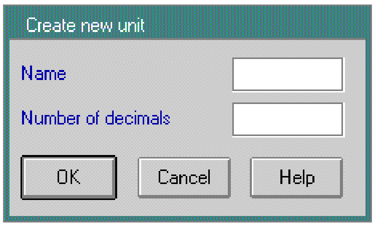
Create curve

Select help curve(s)

01: 96041704:12_Cond1
02: 96041704:12_PressDet
03: 96041704:12_PressACN
04: 96041704:12_CondP
05: 96041704:12_Cond1@01,1dt

Figure 8-14. *Create Curve dialogue box.*

3. If you want to create new unit, click on the **New unit** button and enter the new unit name and number of decimal places the values will receive.



Create new unit

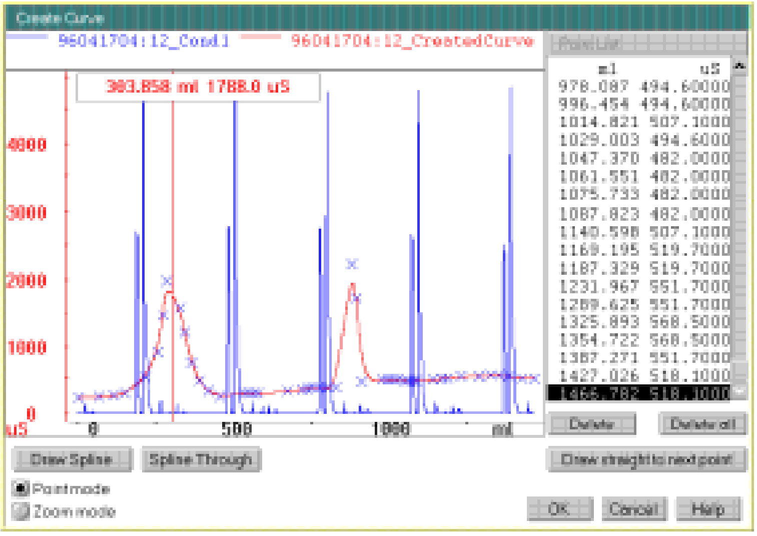
Figure 8-15. *Create new unit dialogue box.*

Click on **OK** to return to the **Create curve** box and again on **OK** when you have made your selections there.

4. With **Point mode** selected, you can use the left button to insert new curve points on the chromatogram. The co-ordinates of each new point are automatically entered into the **Point List**.

   It is possible to precisely define the retention value of the point by activating the **XY** box and selecting the appropriate coloured scale within the box. Using the right mouse button, the cursor bar can be dragged along the x-axis and retention values be read in the active **XY** box. By eye, you can insert a point on the chromatogram at the desired amplitude, at the position of the active bar.

   The right mouse button can also be used to magnify a region of the chromatogram. Selecting **Zoom mode** switches over the mouse button functions, i.e. the left mouse button can be used to drag the cursor bar or perform zoom functions, and the right mouse button inserts points onto the chromatogram.



ml	uS
978.087	494.60000
996.454	494.60000
1014.821	507.1000
1029.003	494.6000
1047.370	482.0000
1061.551	482.0000
1075.733	482.0000
1087.823	482.0000
1140.598	507.1000
1169.195	519.7000
1187.329	519.7000
1231.967	551.7000
1289.625	551.7000
1325.893	568.5000
1354.722	568.5000
1387.271	551.7000
1427.026	518.1000
1466.782	518.1000

__Figure 8-16.__ Create Curve chromatogram window.

5. To delete a point from the **Point List**, double click on the appropriate choice in the list. Alternatively, select the co-ordinates in the list and click on the **Delete** button. To delete all of the points in the list, click on the **Delete All** button.

6. To draw the curve, click on either **Draw Spline** or **Spline Through**. **Draw Spline** creates a smooth curve from the data but does not necessarily pass through every point that you have entered. By

contrast, **Spline Through** creates a curve that passes through all of the data points.

7. In cases where you have created a curve using **Draw Spline**, you may want the curve to pass through a selection of those points currently lying away from the curve. You may force a straight line between two points by selecting the first of the two points in the **Point List** and then clicking on the **Draw straight to next point** command button. This may have to be repeated for several consecutive points to achieve the desired curve.

8. Click on **OK** and save the curve. You can change the curve name from the default, **CreatedCurve**, and also the curve destination.

8.3 Automated evaluation procedures

An evaluation procedure is a recorded sequence of interactive operations in the Evaluation module, which can be executed for automated data evaluation and report generation. It can be used for single chromatograms and for a number of chromatograms in different result files. The concept is analogous to the "macro" facility provided in many word processing and other programs. Evaluation procedures can also be called from methods, making run execution, evaluation and documentation fully automatic. Automation is achieved using the **Procedures** menu.

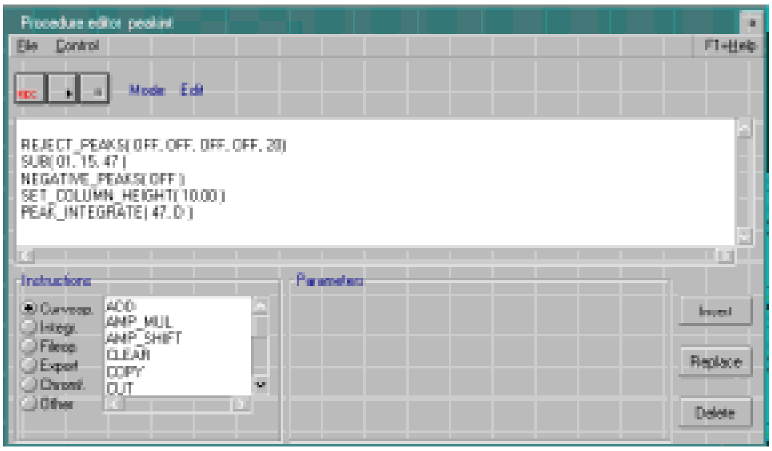
8.3.1 Recording a procedure

1. Open the appropriate results file in the Evaluation module.

2. Record a procedure by clicking on **Procedures:Record on**.

3. Perform the evaluation steps that the procedure is to contain. These steps are recorded as you perform them.

4. When you have finished the appropriate procedures, click on **Procedures:Record off**. The **Procedure editor** dialogue box will open and lists the steps in the procedures you have just created.



```
REJECT_PEAKS( OFF, OFF, OFF, OFF, 20)
SUB( 01, 15, 47 )
NEGATIVE_PEAKS( OFF )
SET_COLUMN_HEIGHT( 10.00 )
PEAK_INTEGRATE( 47, D )
```

● Curveop. ○ Integr. ○ Fileop. ○ Export ○ Chroml. ○ Other

ADD
AMP_MUL
AMP_SHIFT
CLEAR
COPY
CUT

Figure 8-17. *Procedure editor dialogue box.*

5. It is possible to add subsequent steps to the listed procedure by clicking on the **Rec** command button or else select **Control:Record** in the dialogue box menu. Iconise the dialogue box and perform the next evaluation step(s). Stop recording by reopening the iconised dialogue box and clicking on either the **Stop** command button or **Control:Record off**. The new steps are added to the previous procedure.

Note: If a line in the list is highlighted, the new instruction will be inserted after this line.

6. Make any changes required in the evaluation procedure (see Editing evaluation procedures, below).

7. Select **File:Save** or **File:Save as** from the menu bar in the dialogue box. Give the procedure a name. The evaluation procedure is saved within UNICORN OS and is specific to your user name. If you mark the **Save as global procedure**, the procedure will be available for all users. Such procedures are marked with **[Global]** before the name. Even if the results of an evaluation session are not saved, the created evaluation procedure(s) are saved.

8. Choose **File:Exit** from the menu bar in the dialogue box.

Repeat this process from step 2 onwards if you want to create other new procedures.

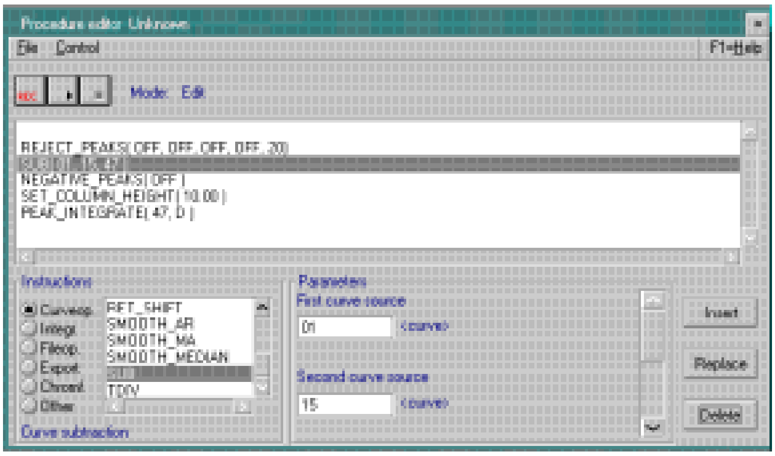
Note: You can also create new procedures by selecting either **File:New:Procedure** or **Procedures:Edit:New**. In either case, the Procedure Editor dialogue box will open and you should than press the **Record** button.

8.3.2 Editing an existing procedure

Evaluation operations are represented by instructions (see Appendix C.4) in the procedure editor. These may be modified to suit specific evaluation needs and be saved for later use.

1. Click on **Procedures:Edit:Open** and select the desired evaluation procedure from the list. The **Procedure editor** dialogue box will open and display the procedure steps.

2. To view a description of the components and parameters of a specific instruction in the procedure, select this from the listed procedure. There are two main fields of interest in the **Procedure Editor** box, namely the **Instructions** field and the **Parameters** field.



Procedure editor: Unknown

```
REJECT_PEAKS( OFF, OFF, OFF, OFF, 20)
SUB( 01, 15 )
NEGATIVE_PEAKS( OFF )
SET_COLUMN_HEIGHT( 10.00 )
PEAK_INTEGRATE( 47, D )
```

Mode: Edit

Instructions

- ● Curveop.
- ○ Integr.
- ○ Fileop.
- ○ Export
- ○ Chrom.
- ○ Other

RET_SHIFT
SMOOTH_AR
SMOOTH_MA
SMOOTH_MEDIAN
SUB
TDIV

Curve subtraction

Parameters

First curve source

01 <curve>

Second curve source

15 <curve>

Figure 8-18. Editing procedures in the Procedure editor dialogue box.

Several radio buttons, e.g. **Curve op**(erations), **Integr**(ation), **File op**(erations), **Export, Chrom**(atogram) **op**(erations) and **Other,** are located in the **Instructions** field which can be individually selected to list a set of related instructions. An instruction selected in a procedure is automatically selected in the **Instructions** field. A definition of a selected instruction is shown in blue text at the bottom left-hand corner of the dialogue box. A list of procedure instructions with descriptions and parameters can be found in Appendix C.3.

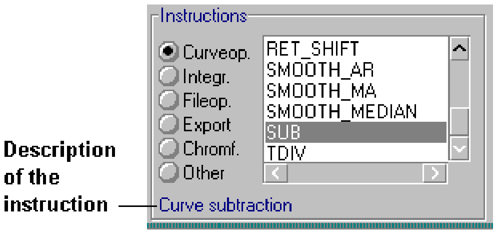


Figure 8-19. *Instructions field in the Procedure editor dialogue box.*

3. In the **Parameters** field the specific parameters contained within the instruction are displayed and clearly identified. Some instructions have many such parameters that can be seen by scrolling up and down using the vertical scroll bar. Other instructions have no parameters at all.

   To edit the parameters of a specific instruction, either type in the new value or choose from the sub-list for a given parameter if such exists. When you have made the desired changes, click on the **Replace** command button. You will now see that the selected instruction in the evaluation procedure is updated in accordance with the new parameters assigned to it.

4. To insert a new instruction line, select the procedure instruction after the position where you want the new instruction to be inserted. Choose an instruction from the **Instructions** field and then enter parameter values. Click on the **Insert** command button. The new instruction will now be added to the evaluation procedure.

5. To remove an instruction from the evaluation procedure, select it in the procedure listing and click on the **Delete** command button.

6. Select **File:Save** or **File:Save as** from the menu bar in the dialogue box. Give the procedure a name.

7. Choose **File:Exit** from the menu bar in the dialogue box.

The **Procedures:Edit** command on the Main menu bar can be used to delete or rename created procedures.

8.3.3 Points to watch

In recording and editing evaluation procedures for automatic evaluation, beware of the following potential pitfalls:

••Make sure that the procedure addresses the right curves. Curves are identified by storage position alone: thus the instruction **ADD (01,02,03)** will try to add curve 01 to curve 02 and store the result in 03, regardless of the contents of 01 and 02. If 03 contains a curve which is not a raw data curve, the existing curve in 03 will be overwritten. If 03 contains a raw data curve, the procedure will stop with an error message. The raw data curves will always occupy the same positions for a given strategy, e.g. UV in position 01. If the operation is not valid when the procedure is run, the procedure will stop at the instruction with an error message. Any subsequent instructions in the procedure will not be executed.

••In calculating a baseline, UNICORN OS suggests default values for the four control parameters (see Section 10.1.2) based on the appearance of the curve. To instruct UNICORN OS to use default values appropriate for the curve every time the procedure is run, choose the **Default** setting in the appropriate fields for the parameters. For example:

```
CALCULATE_BASELINE (01, 06, XXX, XXX, XXX, XXX)
```

can be changed to:

```
CALCULATE_BASELINE (01, 06, DEFAULT, DEFAULT, DEFAULT,
DEFAULT)
```

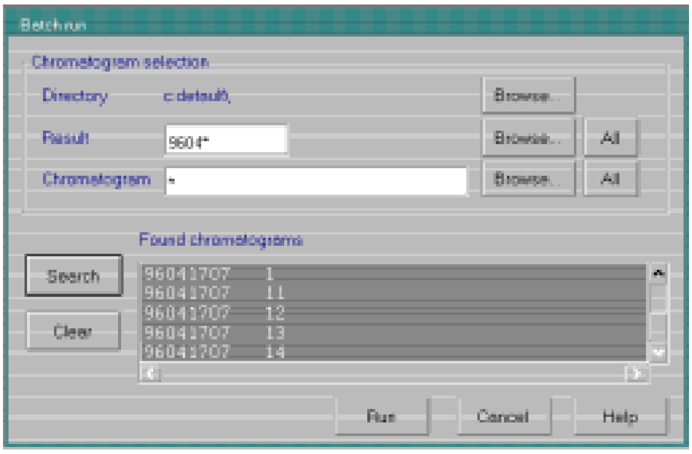
8.3.4 Running evaluation procedures

To run a procedure for a specific chromatogram, first make sure that the desired chromatogram is active. Click on **Procedures:Run** and choose the desired evaluation procedure. Click on **OK** and the procedure runs at once. You can also open the **Procedure editor** dialogue box and choose **Control:Run** or click on the '**Play**' button.

8.3.5 Batch runs

It is possible to apply an evaluation procedure to a designated batch of result files even if they are not open on the Evaluation workspace. It is especially useful, for example, to perform integration with the same parameter settings on many results, or to print a number of results with the same settings. The batch run is done in the background of the Evaluation module and thus the results of the run are not seen. You will, of course, receive any print-outs or report documentation if this was one of the steps in the run procedure (see Section 7.6).

1. Select **Procedures:Batch run**. The **Batch run** dialogue box is displayed.



Directory c:default\

Result 9604*

Chromatogram *

```
96041707    1
96041707    11
96041707    12
96041707    13
96041707    14
```

Figure 8-20. Batch run dialogue box.

2. Select **File:Open related:Curves**. Select the search criteria for the directory, result chromatogram and curve name using the respective **Browse** command buttons. Alternatively, click on the respective **All** button to include all result files, chromatograms and/or curves.

   Wildcard characters, * and ?, can also be used to specify the search parameters (see Section 7.4.1 under *Alternative A*).

3. Deselect those chromatograms that you do not want to be included in the batch run. Click on **OK** to perform the batch run. Any created curves and peak tables will be saved in each result file automatically.

4. To view the results of the batch run on a given result/ chromatogram, open this on the Evaluation workspace.

Note: If one of the result files on which you perform a batch run is the currently opened result file, the created curves will not be shown in the open result file. However, the curves are stored in the original result file with the same name. Therefore, do not select **Save** on the open result file if you do not want to overwrite the curves created during the batch run. It is recommended that you do not perform a batch run on the open result file.

8.3.6 Evaluation procedures and reports

The creation of evaluation procedures, combined with batch runs, is a very useful tool to produce printed documentation simultaneously for many result files, e.g. for a number of scouting runs. This removes the necessity to open/import result files onto the Evaluation workspace. By importing a procedure to a method, printouts can be automatically generated at the end of a run.

1. Begin recording a new procedure by clicking on **Procedures:Record on**.
2. Click on **File:Report** and choose a report format (see Section 7.6).
3. Select **Print** in the **Generate Report** dialogue box as the final instruction. Stop the record function by selecting **Procedures:Record Off**.
4. Save the procedure.
5. Now perform a batch run (see Section 8.3.5) on all the desired result files to get the printed reports. The procedure can also be imported into a method to get automatic printouts at the end of a run (Section 5.6.5).

Note: If the selected report format is changed in **File:Report** the new format will be applied when the procedure is run. If the format is subsequently deleted, the procedure cannot be run.

8.3.7 Placing a procedure on the menu and running

It is possible to place a maximum of 15 created evaluation procedures onto the **Procedures** menu.

Select **Procedures:Menu** and select the evaluation procedure to be added to the menu. Click on **OK**. Activate a chromatogram and select the **Procedures** menu. You will see the procedure that you added to the menu. Select this procedure and it is automatically run for the active chromatogram. The menu addition is remembered in UNICORN OS even if the results of the current evaluation session are not saved.

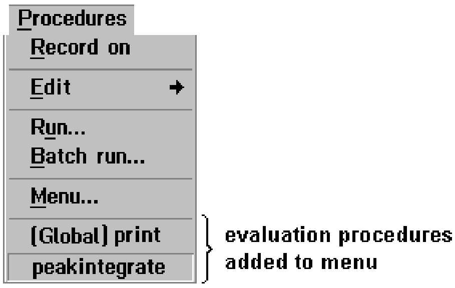


Figure 8-21. *Procedures menu.*

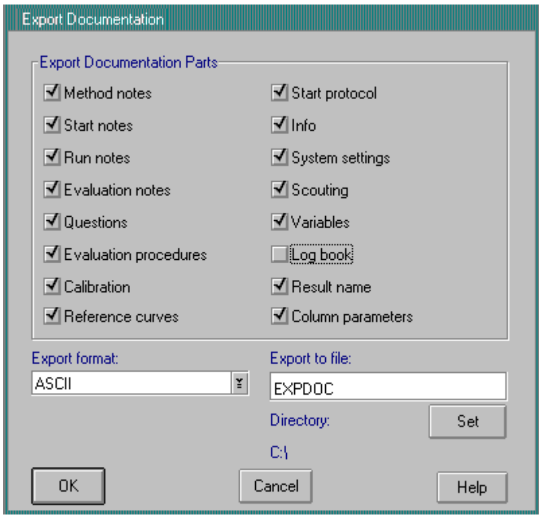
To take away a procedure from the menu, deselect it from the **Procedures:Menu** list.

8.4 Exporting data or curves

Data and curves can be exported to other file formats. Select **File:Export** and then the appropriate sub-menu choice. Alternatively, the **Edit:Copy to clipboard** function can be used, which is the quickest and easiest way to copy a chromatogram into, for example, Microsoft® Word™.

8.4.1 Exporting results

Data can be exported to ASCII (text) and Lotus 1-2-3™ (WKS) spreadsheet formats. The choice of format depends on the subsequent use of the data. If the data is to be inserted into a word processing program, e.g. Microsoft®Word™, it is preferable to export the data in ASCII format. Calculations or diagrams are most easily produced if the data is exported in Lotus 1-2-3 format which can be easily read by most charting and spreadsheet programs, e.g. Microsoft Excel™. Documentation and Evaluation log are exported as text strings, regardless of file format. Peak tables and Curves, however, are exported as text strings in the ASCII format, but as numerical values in the Lotus 1-2-3 format. When exporting peak tables, all possible columns in the table are exported.



Export Documentation

Export Documentation Parts

- ☑ Method notes
- ☑ Start notes
- ☑ Run notes
- ☑ Evaluation notes
- ☑ Questions
- ☑ Evaluation procedures
- ☑ Calibration
- ☑ Reference curves
- ☑ Start protocol
- ☑ Info
- ☑ System settings
- ☑ Scouting
- ☑ Variables
- □ Log book
- ☑ Result name
- ☑ Column parameters

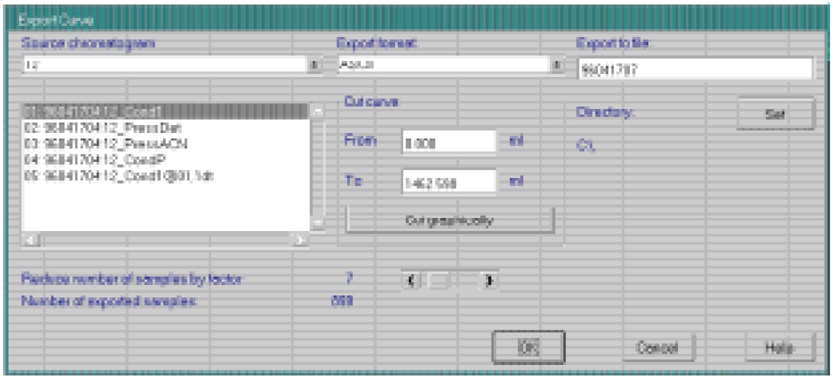
Export format: ASCII

Export to file: EXPDOC

Directory: C:\

Figure 8-22. *Export Documentation dialogue box.*

Curves are exported as two series of numerical co-ordinates referring to time/volume and signal respectively. Only one curve can be exported in each file. When exporting a curve, you may choose to export only a portion of the curve by inserting the limiting retention values directly into the boxes in the **Cut curve** field or by visually selecting the part of the curve using the **CUT GRAPHICALLY** command button option. You may also reduce the number of data points that are exported. For example, reducing the number of data points by a factor of five will export every fifth point to the file.



Export Curve

Source chromatogram: 12

- 01: 9684170412_Cond1
- 02: 9684170412_PressDet
- 03: 9684170412_PressACN
- 04: 9684170412_CondP
- 05: 9684170412_Cond1@01,1dt

Export format: ASCII

Export to file: 96041797

Cut curve

From 0.008 ml

To 1462.558 ml

Directory: C:\

Reduce number of samples by factor 7

Number of exported samples 698

Figure 8-23. *Export Curve dialogue box.*

The file name for the exported data is suggested according to the item being exported, e.g. EXPDOC for Documentation or EXPCRV for Curve. You may change the file name if desired. The extension of the exported file will be **.asc** or **.wks** depending on the file format. By default, files are stored on the root directory on the drive shown in the

Directory field. It is possible to change the destination by pressing the **Set** command button.

8.4.2 Copying results to the clipboard

A related activity is **Edit:Copy to Clipboard** where the contents of the active window are copied to the OS/2 clipboard and can be later easily pasted into other programs, e.g. Microsoft®Word™. Curves and Documentation are copied as bitmaps, while Peak tables are copied as text. In the latter case, only the columns selected in the Chromatogram layout box will be copied.

Introductory material

Methods and Runs

Evaluation

System management

Appendices

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9. Security features

This chapter considers security features in UNICORN OS, under the topics

- access security
- system connection security
- data security
- security recommendations for control stations

9.1 Access security

Operation of UNICORN OS is restricted to authorized users, defined in the system by the system administrator (see Section 12.3). User access may be protected by a password. The minimum password length for users is defined when UNICORN OS is installed (see Section 11.4). In installations where access security is important, passwords should be enforced and should be changed regularly (see Section 12.3.2).

Each user is assigned an access level and a directory access profile, defining the operations that the user can perform and the directories the user is allowed to access (see Section 12.3). For system security, administrative routines such as user and system definition should be permitted only to the system administrator.

Installation of UNICORN OS establishes a default user with full access rights in the system. It is important for system security that this user is deleted when the site-specific users have been created.

9.2 Connection security

To prevent conflicts in System control requests, each system in an installation may have only one control mode connection at any one time.

A running system may be left in a view mode connection and locked with a password (independent of the user's login password) to prevent other users from establishing a control mode connection and interfering with controlling the synthesis process. If the system is left unlocked in a view mode connection, any user may establish a control mode connection to the system.

We recommend that systems are always locked when a user leaves the system. For controlled and locked systems, the responsible user is identified in the System control window for view mode connections. A system which is left unlocked with no control mode connection has no identified responsible user.

Systems may be locked even when they are idle, to allow users to reserve a system for later use.

9.3 Data security

9.3.1 Network communication failure

If the network communication fails while a synthesis is running, any control mode connection on a remote station will lose control of the system and results destined for network drives cannot be saved in their correct directories. In this event, the run continues under control from the local station. Results are saved on the local station with the original result file name in the directory UNICORN OS\LOCAL \FIL\FAILED, from which they can be retrieved after the run is complete. To retrieve results from the FAILED directory:

1. Start UNICORN OS (if it is not already started) on the local station where the synthesis process was run.

2. Log in as a user with access to the FAILED directory.

3. You can now open the result file in the Evaluation module and move it to a suitable location on the network server when network communication is re-established so that it is accessible from remote stations.

Note: Result files are saved directly under the FAILED directory, and are identified by result file name. Files with the same result file name base are distinguished by an incremental serial number, in the same way as result files in any other directory.

Access to the FAILED directory

The system administrator may choose one of two policies concerning access to the FAILED directory:

- ••Grant access to the FAILED directory to all users, so that each user can retrieve his or her own result files. This places the responsibility for retrieving result files and deleting old files from the FAILED directory on the individual users. Note that with this policy any user will be able to examine, copy and move the other users' results in the FAILED directory. This policy has the advantage that the FAILED directory can conveniently be used to temporarily store methods and results from runs performed from the local station when the network is not running.

- Grant access to the FAILED directory only to one or a few users, who share the responsibility for retrieving results files and deleting old files from the FAILED directory. This policy should be used if the installation requires restricted access to users' result files. Note that the user(s) with access to the FAILED directory should also have access to other users' home directories, to be able to copy or move result files to suitable destinations.

In granting access to the FAILED directory, it is sufficient to grant access to C:\UNICORN OS\LOCAL\FIL\FAILED, since this is the path to the FAILED directory on each local station.

9.4 Security recommendations for control stations

Synthesis systems may be controlled without running the user interface modules (the "UNICORN OS application program" on the OS/2 desktop). In a network installation, this situation can arise if a system is controlled from a remote station without starting UNICORN OS on the local station. In a stand-alone installation, the situation can arise if a user quits UNICORN OS after starting a run.

In both cases, it is not apparent from the OS/2 desktop that UNICORN OS control software is actually running, and there is therefore a risk that OS/2 may be shut down and the computer turned off in the belief that it is not in use. To prevent this:

- Do not quit UNICORN OS if you are controlling a system.

- Do not turn off local station computers in a network installation.

- If possible, start UNICORN OS application program on all local stations in a network installation and establish a view mode connection, as an indication that a connected system might be running.

A similar risk arises if UNICORN OS desktop is minimized in installations where this is permitted, since it is not immediately apparent on the OS/2 desktop that the icon is hatched (indicating that the application may be running). For this reason:

- Do not permit UNICORN OS application to be minimized on local computers in a network installation.

- If you minimize the application in a stand-alone installation, leave a clear note on the computer that it should not be turned off.

10. Network setup

UNICORN OS version 1.10 can be installed in a Novell network environment, allowing synthesis systems to be controlled from any PC in the network where UNICORN OS is installed. This chapter describes how to set up the network environment before installing UNICORN OS. You can skip this chapter if you are running UNICORN OS on a stand-alone PC (not connected to a network).

Note: The same version of UNICORN OS must be installed on all computers in the network. Mixing different versions will not work.

10.1 Summary of network setup procedures

Procedures for setting up a networked UNICORN OS environment are summarized in this section and described in more detail in the following sections.

1. Set up a shared hard disk on the server. Make sure that this disk can be accessed with the same drive letter from the PCs where UNICORN OS will be installed.
2. Set up the PC where UNICORN OS is to be installed as a *named pipe server* (Section 10.6). Define a suitable pipe server name for the PC.
3. Reboot the PC, log in to the network and map up to the server disc and directory where UNICORN OS network components are to be installed.
4. Install UNICORN OS for network environment with the installation program for UNICORN OS (Section 11.4).
5. Set up automatic log-in in startup.cmd (Section 10.7).
6. Re-boot the PC and log in to the network.
7. Start UNICORN OS as a user with administration authorization, and set up system definitions and user profiles (see Chapter 12).

10.2 Introduction

Establishing and maintaining a networked UNICORN OS installation requires some understanding of the working of Novell networks and the use of network administration functions. We recommend that the network is maintained by a competent network administrator, who will also be involved in the installation of UNICORN OS software.

The network administrator is not necessarily the same person as the system administrator for UNICORN OS. Once the network is set up, network functions are entirely transparent to UNICORN OS users, and the network administrator does not need to understand the use of UNICORN OS for controlling synthesis systems.

Security is very important in a networked UNICORN OS installation. Three aspects of security can be distinguished, and the responsibility for maintaining security is shared by the network administrator and the system administrator for UNICORN OS:

	Network administrator	*UNICORN OS administrator*
Data storage security (back-up routines)	Back-up routines for server and local disks	Controlled user access to home and shared directories, e.g. placing all home directories on a shared disk to prevent data from being scattered throughout the network
Network access security	Maintenance of user passwords and access rights to shared resources	–
UNICORN OS access security	–	Maintenance of user profiles (see Chapter 12)

10.3 Network organization

Figure 10-1 illustrates how a networked UNICORN OS installation can be organized. There are two kinds of PC where UNICORN OS software is installed:

- •A *local station* is a PC to which synthesis systems are physically connected. UNICORN OS software must be installed on all local stations in the network.

- •A *remote station* is a PC to which no systems are physically connected, but which can control systems over a network link. UNICORN OS software is installed with the **Remote Only** option on remote stations (see Section 11.4).

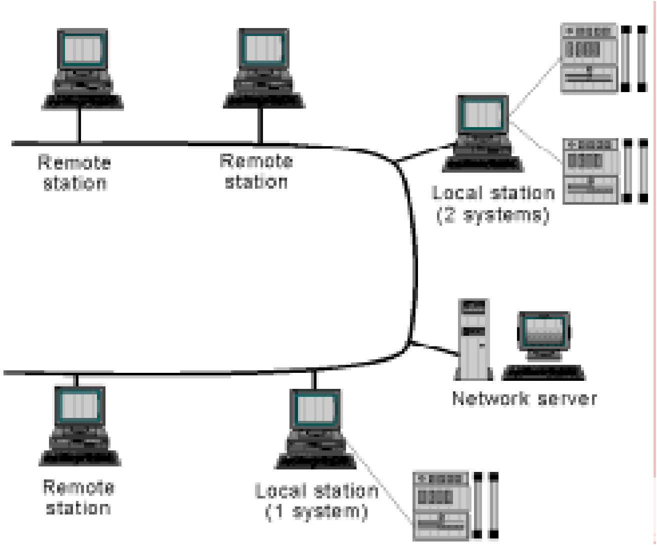


Figure 10-1. *A network installation with 3 synthesis systems and 5 stations (3 remote and 2 local). The systems can be controlled from any computer except the network server.*

The network server requires Novell Netware version 4.02 or higher. The server in a Novell network is a dedicated server, and cannot be set up as either a local or a remote UNICORN OS station.

Synthesis systems in the network can be controlled from either remote or local stations. A local station in a network can also be used as a remote station to control other systems.

Note: If a run is being controlled from a remote station and a network communication error occurs, the run will continue under the control of the local station. Results will be saved in the FAILED directory on the local station (see Section 9.3). A control mode connection can be established on the local station to control the run.

For a synthesis system to be accessible in the network, the local station must be switched on and logged in to the network. The user interface for UNICORN OS does not however need to be started on the local station. System control from a remote station is managed through network level routines which are started in STARTUP.CMD on the local computer.

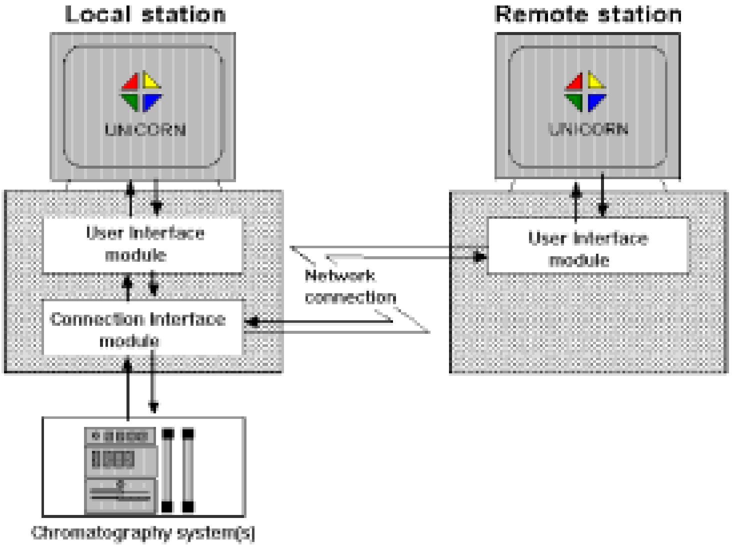


Figure 10-2. The connection interface module in a local station is started automatically and runs separately from the user interface module. This allows systems connected to the local station to be controlled from a remote station without running the user interface module on the local station.

A local station can be used to control the synthesis systems directly connected to the PC without logging in to the network. (The station must however have been logged in to the network once previously so that the necessary files are copied from the network server to the local computer.) This copying is performed automatically. Method and result files stored on network drives will of course not be accessible. For runs performed in this stand-alone mode where the result file is directed to a network drive, the results will be saved in the FAILED directory on the local station (see Section 9.3).

10.4 Network requirements

The following are minimum network requirements for running UNICORN OS in a network installation:

••OS/2 Warp version 3.0 or later.
••Etherlink III network card with 3C5x9.SYS driver (version dated 10th March 1995 or later).
••Novell Netware version 4.02 or later.
••A valid network connection.

10.5 Shared network resources

Installation of UNICORN OS in a network requires access to a shared disk which is accessed through the same logical drive letter from all local and remote stations in the network.

On the Network, a directory should be created by the network administrator in which all UNICORN OS users must be given the minimum Network access rights of Read, Write, Create, Erase, and Filescan. Note that this could be an existing common directory, since the installation program for UNICORN OS is going to create a directory structure under it with the root name UNICORN OS (see Appendix F). Map this drive for all UNICORN OS users to a common drive letter, e.g. Q to match the manual, otherwise any unused letter is fine. All UNICORN OS users must have the same drive mapping letter. Ideally, this mapping should be done to the users log-in script.

10.6 Named pipes

Named pipes are a feature of Novell Netware for OS/2 for peer-to-peer process communication, which permit transmission of real-time data over the network. UNICORN OS stations are set up as named pipe servers so that results are displayed in real time even on remote stations. The pipe server name identifies the station in UNICORN OS system definition (see Section 12.1).

Named pipes are established with the program *Novell Netware Workstation for OS/2. Installation utility version 2.1.* A brief summary of the procedure involved in setting up a named pipe server on a computer where Novell Netware is already installed is given below. Pay particular attention to step 8. Refer to the program documentation from Novell for more details. This procedure must be followed on each local and remote UNICORN OS station in the network.

1. Double-click on the **Novell** icon on the OS/2 desktop and then on the **Install** icon in the **Novell** window.

2. Choose the menu command **Installation:Requester on workstation**.

3. Click on **OK** in the **Set target directory** box.

4. Choose **Only edit config.sys**.

5. Choose the appropriate network driver name (e.g. 3C5x9.SYS for Etherlink III).

6. Choose IPX support on or off as appropriate for your network.

7. Choose **Cancel** in the **Suggested default settings for Autoexec.bat** box if you do not want to change your AUTOEXEC.BAT file.

8. In the **Choose optional protocol** box, select **SPX support for OS/2 sessions**.

   Choose **Remote named pipes support** and **Client and server support**. Specify a name for the pipe server in the **Machine name** field (this is the name that will identify the station in UNICORN OS system definition, see Section 12.1).

   Make a note of the pipe server name for each station. The name is required during installation of UNICORN OS (Section 11.4) and system definition (Section 12.1). Click on **Save**.

Note: Two computers in a network must not be given the same pipe server name. Pay special attention to this if you change the computer to your system.

9 Click on **OK** to save changes to CONFIG.SYS.

10. Choose the menu command **Configuration:This workstation**. Edit NET.CFG to include the following lines

```
PROTOCOL STACK SPX
ABORT TIMEOUT 60000
LISTEN TIMEOUT 12000
VERIFY TIMEOUT 6000
SESSIONS 333

NAMED PIPES
CLIENT SESSIONS 32
SERVER SESSIONS 300
```

11. Save NET.CFG and reboot the computer.

12. Log in to the network and map up to the server disk and directory where UNICORN OS network components are to be installed.

13. Install UNICORN OS (see Chapter 11).

10.7 Startup files

UNICORN OS network stations require some background processes to be started to enable communication over the network. These processes are suitably started from the STARTUP.CMD file in the root directory of the local boot drive. The STARTUP.CMD file is created during UNICORN OS installation.

Each station must also be logged in to the network with the correct logical drive mapping before UNICORN OS is started. A routine should be included in STARTUP.CMD to prompt for username and password to ensure this login.

The example below shows an extract of STARTUP.CMD for a local station with one connected system. The OCI multinet drivers and open connection interface are not required on remote stations. When STARTUP.CMD is first created, the lines marked with an arrow are commented out. Remove the REM text on these lines and edit the MAP line to address the correct server disk and directory before starting UNICORN OS.

```
   REM Example of STARTUP.CMD for a 1-system local station

   REM Prepare Novell Netware for automatic login
➔  CD \NETWARE
➔  login
   REM Edit this line to map the server disk
➔  MAP Q:=SERVER:PROCESS\<dirname>

   REM Storage process for local hard disks
   C:
   CD \UNICORN OS\BIN
   detach storage > nul

   REM OCI multinet drivers
   REM One for each connected system
   detach pmnt_drv 0 > nul
   REM detach pmnt_drv 1 > nul
   REM detach pmnt_drv 2 > nul
   REM detach pmnt_drv 3 > nul

   REM start open connected interface
   REM One for each connected system
   detach OCI 0 > nul
   REM detach OCI 1 > nul
   REM detach OCI 2 > nul
   REM detach OCI 3 > nul
   ...
```

When you make changes to STARTUP.CMD, you must reboot the computer, log-in to the network and restart UNICORN OS for the changes to take effect.

10.8 System and user definitions

Synthesis systems accessible from UNICORN OS network stations are defined by the system administrator for UNICORN OS with the **Administration: System** command in the Main menu (see Section 12.1). Parameters that must be defined for each system in a network include

- ••the system name to help in identifying the system in the network (e.g. 'Michaels OligoPilot' or 'OligoPilot #2')
- ••the strategy associated with the system
- ••the local station name (i.e. the pipe server name on the PC to which the system is physically connected)
- ••the control unit number on the local station (each local station can handle up to 4 systems).

Each UNICORN OS user has a user profile which defines access rights, data storage locations etc. These parameters are defined by the system administrator for UNICORN OS using the **Administration:User Setup** command in the Main menu.

Both the system and user definitions are stored on the network server and apply globally throughout the network. Changes to system and/or user setup can be made from any station in the network.

11. Installation

This chapter describes how to install UNICORN OS hardware and software.

11.1 Installation summary

The following installation procedures are required before UNICORN OS systems can be used:

1. Set up the network environment (for network installations only) (Chapter 10).
2. Install UNICORN OS hardware and software (this chapter).
3. Set up system definitions for the attached synthesis systems (Section 12.1).
4. Define access levels for the installation (Section 12.2).
5. Define users with home directories and access profiles (Section 12.3).
6. Check the system settings for the attached systems (Chapter 13).

11.2 System requirements

11.2.1 Hardware requirements[1]

Computer type:	IBM compatible PC, Compaq, with at least one free full-length expansion slot.
Operating system:	IBM OS/2 Warp (registered trademark of IBM Inc.) version 3.0 or later.
CPU:	Pentium/75 MHz or higher.
RAM minimum:	24 Mb.
Hard disc recomm.:	1 Gb
Floppy disc drive:	3.5” 1.44 Mb
Communication:	Etherlink III network card with 3C5x9.SYS driver (version dated 10th March 1995 or later).
Network support:	Novell Netware (registered trademarks of Novell Inc.) version 4.02 or later. A valid network connection.
Printer or plotter:	As supported by OS/2 Warp operating system.
Mouse:	Microsoft mouse or compatible.
Monitor:	Colour SVGA (Compaq QVision)

[1] Hardware recommendations apply in February 1997. Contact your local sales office for details of the latest hardware recommendations.

The MCU for communication between UNICORN OS and the synthesis system(s) is supplied as part of the system(s) and is not included with UNICORN OS. The MCU is available for (E)ISA- and MCA-bus computers.

11.2.2 Software requirements

IBM Operating System OS/2 Warp version 3.0 or later.

Novell NetWare version 4.02 or later for network installations.

Network installation

UNICORN OS can be installed in network configurations using Novell NetWare version 4.02 or higher. Computers to which synthesis systems are physically connected are termed *local computers.* Computers from which systems are to be controlled over the network are termed *remote computers*. Systems can also be controlled remotely from a local computer. The table below summarizes the installation requirements for UNICORN OS on computers in the network:

Computer	*Hardware*	*Software*
Local	MCU	UNICORN OS
Remote	–	UNICORN OS
Network server	–	UNICORN OS (installed automatically)

You must always log in to the network before installing or setting up UNICORN OS software.

See Chapter 10 for full details of network setup and administration.

11.3 Hardware installation

In most cases, an installation of your system will be performed by Amersham Biosciences authorized personnel. If your system is not pre-installed, then follow the steps below to install the main control unit (MCU) expansion card in your PC. This procedure is only required in stand-alone installations (i.e. systems not connected to a network) and on local computers in network installations.

1. Turn off the power to your PC and unplug the mains cable.

2. Open the PC cover. Refer to your PC documentation if you are not sure how to do this.

3. Locate an empty full length expansion slot.

4. Take the MCU card out of the anti-static bag. Handle the card by its edges, and avoid touching the electronic components as far as possible. Discharges of static electricity can permanently damage electronic components on the card. If you are working in a room where static electricity tends to build up, discharge any electricity from your body by touching an earthed metal surface (e.g. a water tap or radiator) before handling the card.

   Install the MCU card into the expansion slot. Your PC documentation should describe how to fit the card: if you are uncertain, contact your computer supplier.

4. Close the cover on your PC.

5. Connect the MCU card to the liquid handling module CU connector using the communication cable provided. Additional liquid handling modules up to a total of four may be connected in series. *Note:* The connection to the MCU card is made with a 15-pin connector for ISA-bus computers (Compaq) and a 9-pin connector for MCA-bus computers (IBM PS2).

11.4 Software installation

UNICORN OS will normally be installed by your Amersham Biosciences representative. Follow the instructions below if you need to install the program yourself. The software must be installed on all stations in a network.

11.4.1 Installing UNICORN OS for the first time

The installation procedure described below assumes that the operating system OS/2 is correctly installed on your computer. Refer to the operating system documentation for details. For network installations, the network must be correctly set up as described in Chapter 10.

Note: System fonts must be installed in OS/2 for correct functioning of UNICORN OS.

UNICORN OS is supplied on 3.5" high density diskettes. Files on the distribution diskettes are compressed, and cannot simply be copied to the hard disk. The installation procedure described below creates the required directory structure on the hard disk and decompresses the distribution files. Do not attempt to decompress the distribution files using any other file decompression utility.

Before installing UNICORN OS, use the OS/2 DISKCOPY command to make working copies of the distribution diskettes and store the originals in a safe place. See your OS/2 documentation for further details of how to use DISKCOPY. Amersham Biosciences does not undertake to replace lost original diskettes without charge.

Follow the procedure below to install UNICORN OS. For a network installation, follow this procedure on each computer in the network. You can quit the installation at any point by pressing function key F3 or clicking on the **Exit** button. If you do this, however, the installation will be incomplete and the software cannot be used.

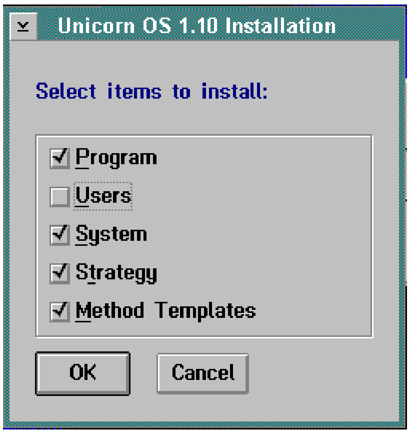
1. For network installations, log in to the network and check that you are mapped up to the server disk and directory where UNICORN OS network components are to be installed. To view and change your mapping, click on the **Novell** icon, run **Netware tools** and select **Tools:Disk drives**.

2. Insert diskette no. 1, labelled Installation, in diskette station A.

3. Open an OS/2 command prompt window on the OS/2 desktop. Command prompts can be found by default in a **Command prompt** folder in the **OS/2 System** folder (you may also have set up command prompt icons elsewhere on the OS/2 desktop).

4. Enter the command `a:install`.

5. Select the items to install then click on **OK.**



Unicorn OS 1.10 Installation

Select items to install:

☑ Program
□ Users
☑ System
☑ Strategy
☑ Method Templates

Stand-alone installation

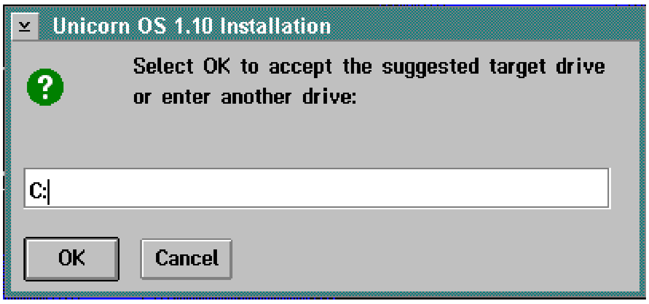
- ••Check all components.

Network installation

- ••**Program** must be checked on all stations, both local and remote.
- ••**System** must be checked on all local stations but should not be checked on remote stations.
- ••**Users** should be checked *for the first installation only*. If **Users** is checked during later installation on other computers in the network, any users already defined will be deleted and only the **default** user will be available.

- ••**Strategy** and **Method Templates** should be checked for each new strategy to be installed (usually when a new system is installed on a local computer). Each strategy need only be installed once. **Method Templates** should not be checked for BioProcess System installation.

6. Enter the disk where the program is to be installed and click on **OK**. This should be a physical disk (usually C:) in the computer where you are installing UNICORN OS, not a network disk.



Unicorn OS 1.10 Installation

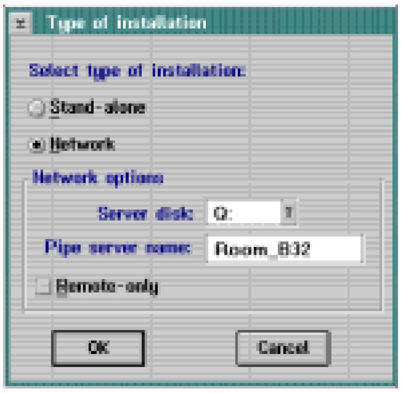
Select OK to accept the suggested target drive or enter another drive:

C:

7. Choose whether the installation is stand-alone or network. For a stand-alone installation, the **Network options** settings are ignored. For a network installation, choose the server disk from the pull-down list in the **Server disk** field and enter the pipe server name of the station (see Section 10.6) in the **Pipe server name** field. The pipe server name can be found if necessary in the CONFIG.SYS file on the line:

```
RUN = C:\NETWARE\NPDAEMON.EXE <pipe server name>
```

For installation on a local station, make sure that **Remote-only** is not checked. For installation on a remote station (i.e. a computer to which no synthesis systems are physically connected), check **Remote-only**.



Type of installation

Select type of installation:

○ Stand-alone

● Network

Network options

Server disk: Q:

Pipe server name: Room_B32

□ Remote-only

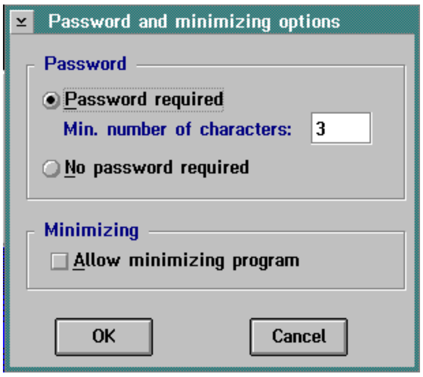
When you perform a network installation, the necessary parts of UNICORN OS software will be copied automatically to the network server disk.

Note: If you perform a stand-alone installation and later want to connect the system to a network, you must re-install UNICORN OS software with the appropriate settings.

8. Choose the minimum number of characters required for passwords (this number applies to both login and locking passwords). Alternatively, if you do not require password protection, check **No password required**. With this setting, users can be defined with or without passwords.

Note: In a network installation, make sure that you enter the same password settings on each station in the network.

Check **Allow minimizing program** if you want to be able to minimize the UNICORN OS window on the OS/2 desktop.



Password and minimizing options

Password

● Password required

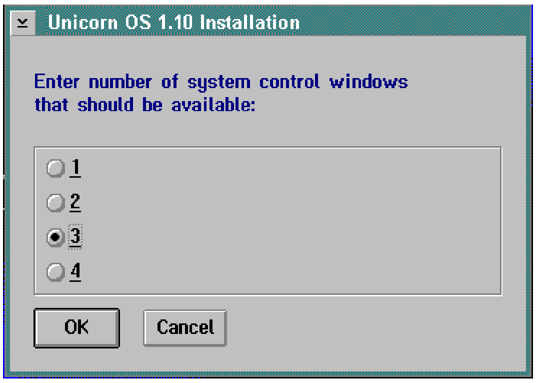
Min. number of characters: 3

○ No password required

Minimizing

□ Allow minimizing program

9. Choose the number of System control windows that should be available in the installation (maximum 4).



Unicorn OS 1.10 Installation

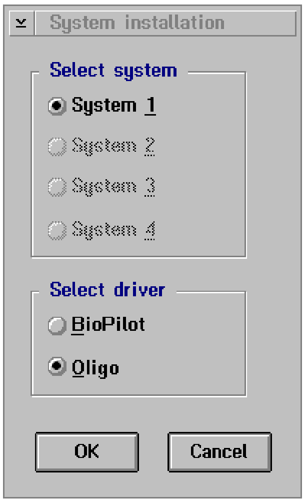
Enter number of system control windows that should be available:

○ 1
○ 2
● 3
○ 4

The installation will now start. If global procedures and report formats exist already (e.g. in a network), you will be asked if you want to replace the corresponding files. Normally you should answer **No**. If you answer **Yes**, all existing global procedures and report formats will be deleted and replaced by the defaults.

Change to diskette no. 2 and 3 when prompted to do so.

10. If you have chosen to install **Systems**, choose the system to install and the driver. For OligoPilot II, select **Oligo**. When the software is installed for the first time, the choice is made automatically. You can choose the system to install if you have more than one system already present and you wish to reinstall for one of the existing systems.



System installation

Select system

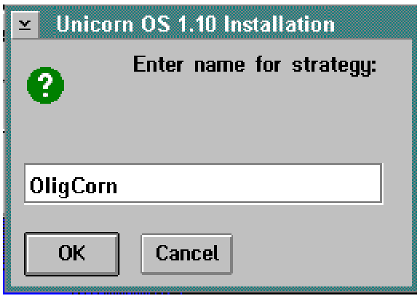
● System 1
○ System 2
○ System 3
○ System 4

Select driver

○ BioPilot
● Oligo

11. Choose to install additional systems until you have installed all systems physically connected to the computer. (The number of systems is not related to the number of System control windows installed in step 9). If you installed systems with different drivers, make a note of which driver was selected for each system number. When you later define systems in Main menu **Administration: System**, the control unit number for each system must correspond to the correct driver.

12. If you have chosen to install **Strategy** and/or **Method Templates**, insert the Strategy/templates disk when prompted to do so. Enter a suitable name for the strategy.



Unicorn OS 1.10 Installation

Enter name for strategy:

OligCorn

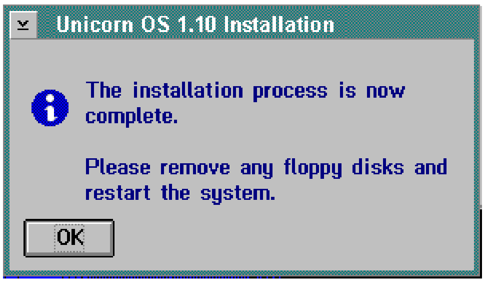
Templates are installed automatically together with the strategy.

13. Choose to install additional strategies if you have more than one configuration of synthesis system.

Note: Strategies are installed independently of systems. A strategy is assigned to a system when the system is defined (see Section 12.1).

14. After installation is complete, you must remove the diskette and restart the computer for the installed software to be properly configured. For computers in a network, make the required changes to STARTUP.CMD (see Section 10.7) before restarting the computer.

If 3 or 4 systems are connected to a computer, either physically or through the network, the line THREADS=256 in the CONFIG.SYS file must be changed to THREADS=512.



Unicorn OS 1.10 Installation

The installation process is now complete.

Please remove any floppy disks and restart the system.

To restart the computer, click with the right mouse button on the OS/2 desktop and choose **Shutdown** from the desktop menu. When the shutdown procedure is complete, press **Ctrl-Alt-Del** or turn the power off and on again to restart the computer.

11.4.2 Installing selected software components after the initial installation

If your UNICORN OS installation should be damaged (due to accidental file deletion or hard disk failure) or if you want to install additional systems, strategies or templates, you can use the installation program to re-install selected parts of the software. The installation program detects the presence of existing UNICORN OS files, and suggests the existing disk for installation. Check some or all of **Users**, **Program**, **System**, **Strategy** and **Method templates** according to which part(s) you wish to install. The respective parts will be copied into the existing directory structure.

Note: For network installations, remember to log in to the network before installing any UNICORN OS software components.

Users

Check the **Users** button to re-install the default user if the existing user definitions are damaged. Any other users defined in the system (including users installed from other stations in a network installation) will be deleted, but method and result files will not be erased from the disk. You can regain access to these files by redefining users with appropriate directory access (see Section 12.3 for further details of defining users).

For example, assume that the system has a user called ARNOLD with access to the home directory ARNOLD. To regain access to Arnold's files after installing the **Users** option, simply define a new user with ARNOLD as the home directory.

Users are installed on the network server in a network installation.

Program
Before reinstalling the program, you must delete STARTUP.CMD from the root directory on drive C: and reboot the computer.

Check the **Program** button to reinstall UNICORN OS. This will not affect any existing method or result files in the system.

The program is always installed locally, even in network installations.

In network installations, remember to make the required changes to STARTUP.CMD after reinstalling the program (see Section 10.7).

System
Check the **System** button if you are installing a new or additional system on a stand-alone computer or a local station in a network.

Systems are only installed on local stations in a network installation.

Strategy
Check the **Strategy** button to re-install system strategies or to install additional strategies. Accept the suggested name or enter a new name for the strategy. This will not affect any existing method or result files in the system.

Strategies are installed on the network server in a network installation.

Method templates
Check the **Method templates** button in the installation program to re-install method templates or to install method templates for a new strategy. Normally, templates are installed together with strategies. You will be asked to enter the strategy name to which the method templates correspond if you are not installing a strategy at the same time.

Templates are installed on the network server in a network installation.

11.4.3 Installing software upgrades

Instructions for upgrading UNICORN OS installations to new versions will be provided with the upgrade software.

12. Administration

There are three main aspects of administration of a UNICORN OS installation:

••System administration

••Access level and user administration

••Network administration where appropriate.

System administration concerns maintenance of software aspects of the installation, including definition of connected systems and monitoring of system usage (audit trails). These activities are described in the present chapter. System administration duties may also include routine monitor calibration (Section 6.6).

Access level and user administration concerns authorization of access to the system, and should (at least in larger installations) be the responsibility of one person or a small group. These activities are described in the present chapter.

In a network installation, maintenance of the network functions will normally be carried out by the computer staff responsible for the company's network. Aspects of network administration relevant to UNICORN OS are considered in Chapter 10.

After installation, the following operations should be performed in UNICORN OS by the administrator before other users can use the program:

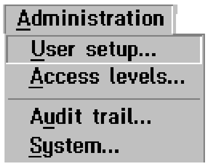
1. Set up system definitions for the synthesis systems (Section 12.1).
2. Define access levels for the installation (Section 12.2).
3. Define new users with home directories and access profiles (Section 12.3).

Note: These operations can be performed on any station in a network installation. It is however important that the administrator is logged in to the network on the station being used so that the changes will apply globally throughout the network.

12.1 System definitions

System definitions set up the synthesis systems which are connected directly to the local computer in UNICORN OS installation. This must be done for each new system installed. In a network installation, these definitions must be set up for each local computer in the network, but the actual set-up operations can be performed on any computer. Rights of access to system are controlled at the user administration level (Sections 12.2 and 12.3).

To manage system definitions, choose **Administration:System** from the Main menu. To use this menu command, you must have **Audit trail/System setup** authorization (see above).



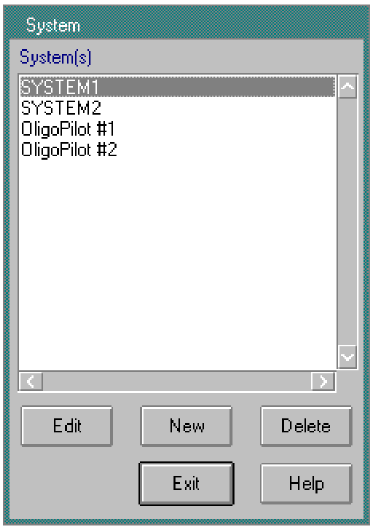
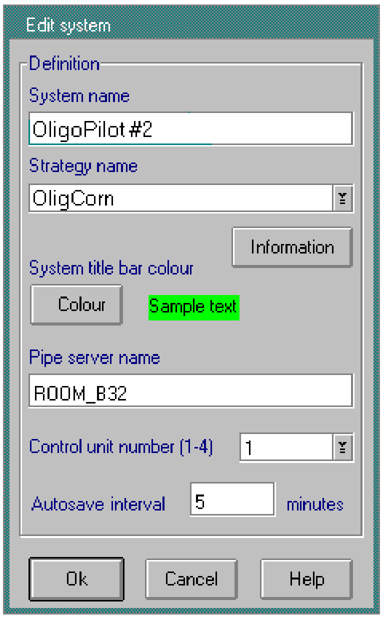


Figure 12-1. System definition dialogue box.

12.1.1 Defining and editing systems

To define a new system, click on **New** in the **System** dialogue box. To edit an existing system, choose the system in the **System** dialogue box and click on **Edit**. Change parameters as required.



Edit system

Definition

System name: OligoPilot #2

Strategy name: OligCorn

System title bar colour: Sample text

Pipe server name: ROOM_B32

Control unit number (1-4): 1

Autosave interval: 5 minutes

Figure 12-2. *Dialogue box for defining and editing systems.*

1. Enter the system name in the **System name** field. The system name can be set only when defining a new connection, and cannot later be edited since users' access rights are linked directly to the system name. Names can be up to 30 characters long.

2. Select a strategy for the system from the pull-down list in the **Strategy name** field. Available strategies are determined when UNICORN OS is installed (see Section 11.4). If you have several strategies installed, make sure that the selected strategy is appropriate for the chromatographic system being defined. Click on **Information** in the **Strategy** dialogue box to display information about the selected strategy.

3. Set the system title bar colour if desired by clicking on **Colour**. You can choose any combination of bar colour and text colour from the palettes in the dialogue box that appears. The selected colours will be used in the title bar for System control windows for the particular system, and can provide a quick identification of which systems are connected to the different System control windows.

4a. Stand-alone installations
For stand-alone installations, the **Pipe server name** field is a dummy field with default value **LOCAL**. Leave this value unchanged.

4b. Network installations

In the **Pipe server name** field, enter the pipe server name of the computer to which the system being defined is physically connected. See Section 10.6 for information concerning pipe server names.

The pipe server name can be found if necessary in the CONFIG.SYS file on the line

```
RUN = C:\NETWARE\NPDAEMON.EXE <pipe server name>
```

5. Select the control unit number (1-4) in **Control unit number**. This is the physical connection number for the synthesis system on the local computer (see Chapter 11). If you only have one system physically connected to the local computer, this number should be 1.

6. Enter a value in the **Autosave interval** field if you want UNICORN OS to save a copy of the result file at preset intervals during a run. This minimizes loss of data in the event of a computer failure. The recommended interval for most systems is 5 minutes. A shorter interval may slow down the user interface response. The control functions in UNICORN OS performance will however not be impaired.

Note: Normally, you should define systems before defining users. If you add system definitions after you have defined users, remember to grant access to the new systems to the appropriate users (see Section 12.3).

12.1.2 Deleting system definitions

To delete a system definition, select the definition in the **System(s)** list and click on **Delete**. A system definition can only be deleted if the system is idle and no user has a control mode connection to the system.

12.1.3 Changing strategies

You can change the strategy assigned to a system provided that the system is idle and no user has a control mode connection to the system. Choose **Administration:System,** check the system in the **System** dialogue box and click on **Edit**. Choose the required strategy and click on **OK**.

Remember that the arrangement of tubing, pumps, columns etc. may need to be changed on the synthesis system if you change the strategy. An attempt to control a system using the wrong strategy may cause malfunction and damage to the synthesis system.

12.2 Access levels

Access to UNICORN OS software is controlled by username and password authorization (see Sections 12.3). Within the program, each authorized user is assigned an access level within the system which determines which functions the user can perform:

12.2.1 Defining access levels

Up to 10 different access levels can be defined. Initially, all levels are the same with access to all functions.

To edit an access level, choose **Administration:Access levels** from the Main menu and choose the level you wish to edit. To use this menu command, you must have **User setup/Levels** authorization (see below). The levels are named **Level 1 - Level 10** by default: to change the name of a level, simply type a new name in the **Level name** field. Check the items to which users at this level are to have access, and click on **OK**. If you change the definition of a level to which users are already assigned, the changes will apply to all users at this level.

At least one access level must have **User setup/Levels** authorization. UNICORN OS will not allow you to remove this authorization from all levels.

The authorization items are:

- **Method Editor**
  Required for using the Method editor for creating and editing methods (Chapters 4 and 5).

- **Evaluation**
  Required for using the Evaluation module for processing result data (Chapters 7 and 8).

- **User setup/Levels**
  Required for defining and changing access levels and user.
  IMPORTANT: We recommend that only one user in an installation or network is assigned this access.

- **Audit trail/System setup**
  Required for examining the audit trail and for defining connected systems (Section 12.4).
  IMPORTANT: We recommend that only one user in an installation or network is assigned this access.

- **Delete, move - Home only**
  Required for deleting and moving files and directories within the user’s home directory (Sections 3.2.5 and 3.2.6). Does not authorize these operations on other directories.

- **Delete, move**
  Required for deleting and moving files and directories outside the user’s home directory (Sections 3.2.5 and 3.2.6). Also authorizes these operations within the home directory.

- **Copy files**
  Required for copying files (Section 3.2.5). The user must have access to both the source and target directories for moving or copying between directories.

- **Confirm**
  Required for authorized confirmation of answers to start protocol questions (Section 5.6.2).

- **Unlock locked system**
  Permits a user to unlock locked systems by providing the user’s own log-in passwords (locked systems can normally only be unlocked using the locking password, see Section 6.5.3). We recommend that this authorization is restricted to a few users in an installation. The user who locks a system does not require this authorization to unlock the same system.

- **Run methods**
  Required for starting methods (Section 6.1).

- **Manual interaction**
  Required for issuing manual commands in System control (Section 6.3).

- **Pause**
  Required for pausing a running process with the PAUSE button in System control (Section 6.3.1). The PAUSE instruction in methods does not require explicit authorization.

- **Calibrate/tune**
  Required for using the **Calibrate** and **Tune** commands in System control (Section 6.6).

- **System settings**
  Required for changing system settings with the **Settings** command in System control (Chapter 13). Any user may view the system settings, but this authorization is required to make changes to the settings.

- •**Edit sequences**
  Required for using the sequence editor.

- •**Run sequences**
  Required for running sequences.

- •**Edit global lists**
  Required for saving a method as a method template, an evaluation procedure or a report format as globally available. Also required for deleting method templates, global procedures or global report formats.
  We recommend that this authorization is restricted to only one user in an installation.

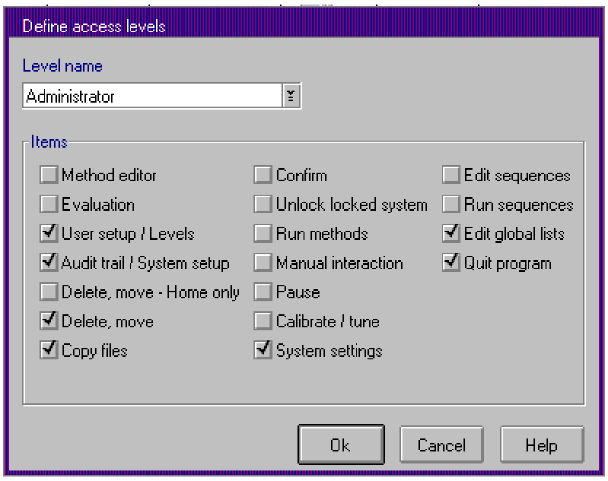
- •**Quit program**
  Required for ending a UNICORN OS session with the **Quit** command in the Main menu.

12.2.2 Access level examples

Below are some examples illustrating the way access levels might be used in a multi-user installation.

System administrator
The administrator has special responsibility for maintaining user, system and audit information and for file management in the PC (directory structure, backup routines, etc.). The administrator may not edit or run methods or sequences, issue manual instructions, calibrate monitors or change the system configuration.

> *Important!*
> We recommend that only one user in an installation or network is assigned system administrator rights. If several users can change user definitions and system connections, confusion can rapidly follow.



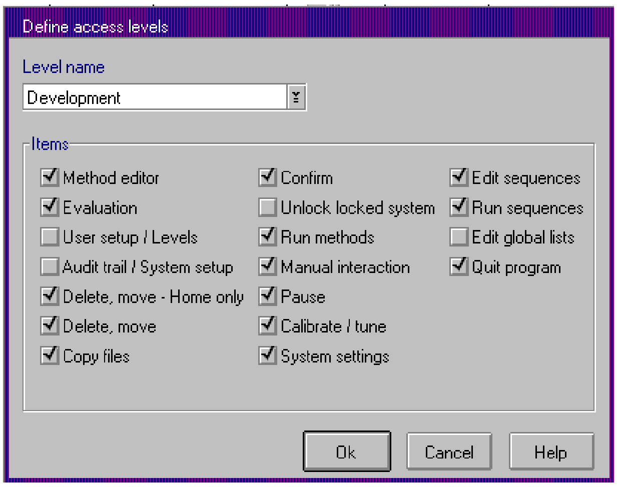
Level name: Administrator

Items

□ Method editor
□ Evaluation
☑ User setup / Levels
☑ Audit trail / System setup
□ Delete, move - Home only
☑ Delete, move
☑ Copy files
□ Confirm
□ Unlock locked system
□ Run methods
□ Manual interaction
□ Pause
□ Calibrate / tune
☑ System settings
□ Edit sequences
□ Run sequences
☑ Edit global lists
☑ Quit program

Figure 12-3. *Suggested authorization profile for the system administrator.*

Development staff
Developers need to be able to edit and run methods and sequences, issue manual instructions, configure system parameters, calibrate monitors and evaluate data. They may also copy, move or delete files.



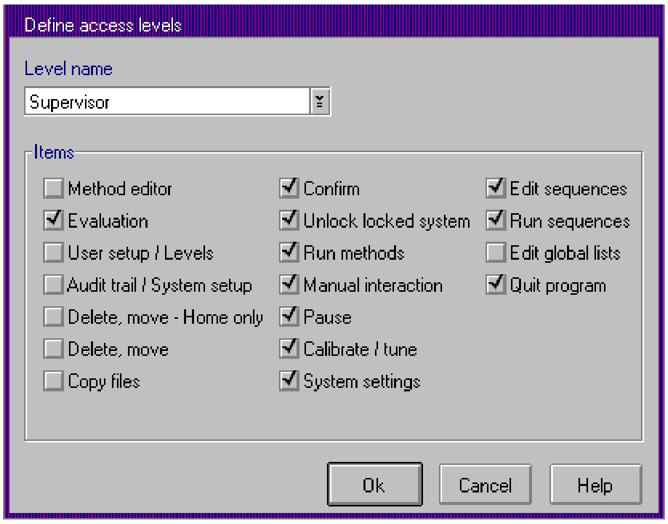
Level name: Development

Items

☑ Method editor
☑ Evaluation
□ User setup / Levels
□ Audit trail / System setup
☑ Delete, move - Home only
☑ Delete, move
☑ Copy files
☑ Confirm
□ Unlock locked system
☑ Run methods
☑ Manual interaction
☑ Pause
☑ Calibrate / tune
☑ System settings
☑ Edit sequences
☑ Run sequences
□ Edit global lists
☑ Quit program

Figure 12-4. *Suggested authorization profile for development staff.*

Process supervisors
Supervisors may pause a method and issue manual instructions as well as start methods and sequences. Supervisors are also allowed to calibrate monitors, configure system parameters, unlock running processes, evaluate run data and quit UNICORN OS.



Define access levels

Level name

Supervisor

Items

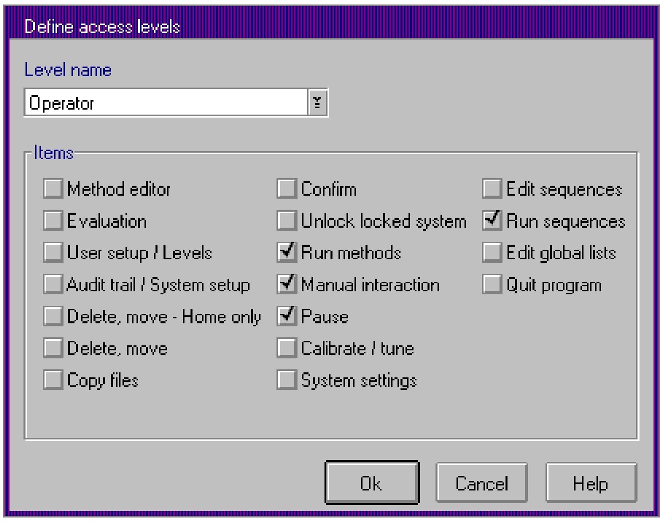
☐ Method editor
☑ Evaluation
☐ User setup / Levels
☐ Audit trail / System setup
☐ Delete, move - Home only
☐ Delete, move
☐ Copy files

☑ Confirm
☑ Unlock locked system
☑ Run methods
☑ Manual interaction
☑ Pause
☑ Calibrate / tune
☑ System settings

☑ Edit sequences
☑ Run sequences
☐ Edit global lists
☑ Quit program

Figure 12-5. *Suggested authorization profile for process supervisors.*

Process operators
Process operators are allowed to run and pause methods and sequences but may not perform any other operations.



Define access levels

Level name

Operator

Items

☐ Method editor
☐ Evaluation
☐ User setup / Levels
☐ Audit trail / System setup
☐ Delete, move - Home only
☐ Delete, move
☐ Copy files

☐ Confirm
☐ Unlock locked system
☑ Run methods
☑ Manual interaction
☑ Pause
☐ Calibrate / tune
☐ System settings

☐ Edit sequences
☑ Run sequences
☐ Edit global lists
☐ Quit program

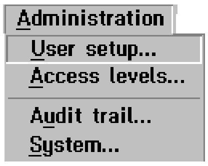
Figure 12-6. *Suggested authorization profile for process operators.*

12.3 User administration

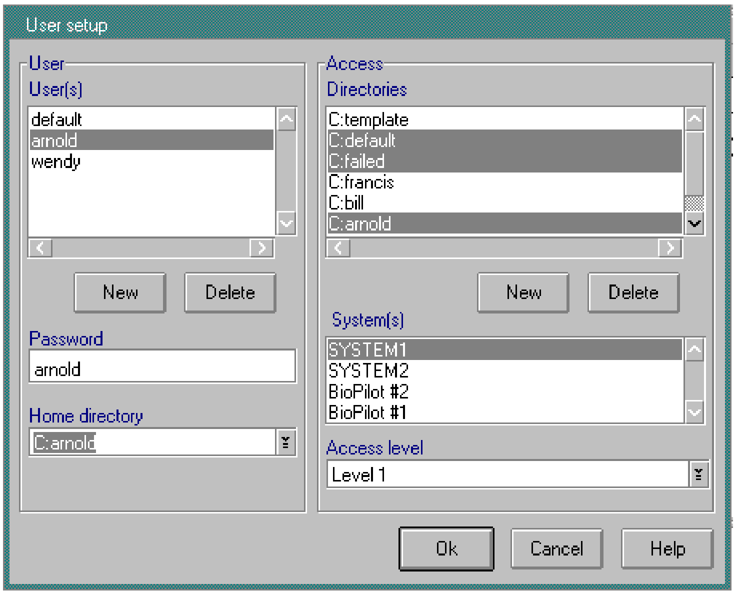
All UNICORN OS users are identified by a username and password. A new installation is provided with a default user (username:**default**, password:**default**). This user provides unrestricted access to all UNICORN OS functions.

> *Important!*
> As part of the installation procedure, new users should be created with passwords and restricted access rights as required. The default user should be deleted or redefined to prevent unauthorized access to the system.

Maintenance of user authorization information is the responsibility of the system administrator. In a newly installed system, log in as user **default**.



To define, edit or delete users, choose **Administration:User setup** from the Main menu. To use this menu command, you must have **User setup/Levels** authorization (see above). All user administration is performed from this dialogue box.



User(s): default, arnold, wendy

Directories: C:template, C:default, C:failed, C:francis, C:bill, C:arnold

System(s): SYSTEM1, SYSTEM2, BioPilot #2, BioPilot #1

Password: arnold

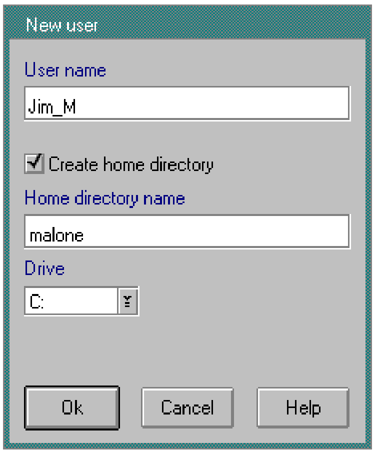
Home directory: C:arnold

Access level: Level 1

Figure 12-7. The User set-up dialogue box.

12.3.1 Defining new users

1. To define a new user, click on **New** and enter the username in the dialogue box. The username can be up to 15 characters long and can contain any combination of letters, digits or other printable characters.



New user

User name: Jim_M

☑ Create home directory

Home directory name: malone

Drive: C:

Figure 12-8. *Dialogue box for defining a new user.*

2. To create a new home directory for this user, check the **Create home directory** box and enter the directory name and drive in the appropriate fields. If you choose to create a home directory on a network drive, make sure that the drive is always accessible. Click on **OK** to create the user and return to the **User setup** dialogue box.

Note: A home directory can always be created on a network drive even if UNICORN OS is not installed for network control. The computer only need to be connected and logged in to the local network.

In general, each user should have a separate home directory.

> *Important!*
> In a network installation, always create home directories on a network drive which is accessible from all computers. If you create a home directory on the C: (local) drive, it will only be accessible from the computer on which it was created.

3. Enter the user's password in the **Password** field. The minimum number of characters in the password is defined when UNICORN OS is installed (see Section 11.3). The program may also be

installed without password protection. The case of letters in passwords is not significant.

> *Warning!*
> Leaving the password as **default** can constitute a serious security risk.
>
> All user passwords are visible in the **User setup** dialogue box. For security reasons, make sure that access to this function is restricted.

4. Select a home directory from the pull-down list in the **Home directory** field. (You may choose any directory from this list, even if you created a home directory in the **New user** dialogue box.)

5. In the **Access** panel, select other directories to which the user will have access from the **Directories** list. Selecting a directory here will give the user access to all files and subdirectories therein. Directories that are not selected in this list will not be visible in the methods or results panel of the Main menu.

> Note: All users should be given access to the FAILED directory on each local station in a network installation. This will ensure that users can access results saved in the FAILED directory in the event of a network communication error.

6. Select the system(s) to which the user will have access from the **System(s)** list and the access level of the user from the **Access level** list.

You can continue to define new users as long as the **User setup** dialogue box is open. Click on **OK** to close the dialogue box. If you close the box by clicking on **Cancel**, all changes made since you opened the dialogue box will be lost.

12.3.2 Changing user passwords

Every user can change his or her own password with the **User:Password** command in the Main menu. Enter the old password and the new password twice in the appropriate fields. Passwords are not displayed explicitly in this dialogue box. The password will not be changed if either the old password is incorrect or the two copies of the new password differ from each other.

In addition, a user with **User setup/Levels** access can change the password for any user. To change a password for another user, open

Administration:User setup, select the user and enter a new password in the **Password** field (see Section 12.3.1).

Change passwords regularly and avoid obvious passwords like “secret” and “open_sesame” for maximum security.

If you forget the password for the only user with **User setup/Levels** access, you must re-install the default user as described in Section 11.4.2.

12.3.3 Viewing and changing user definitions

To view the setup for any user, click on the user name in the **User setup** dialogue box. To change the user definitions, make changes in the dialogue box fields as appropriate and click on **OK**.

12.3.4 Deleting users

To delete a user, select the user from the username list and click on **Delete User**. You may delete all users except the last user with **User setup/Levels** access. This ensures that at least one user has the right to perform administration functions.

Deleting a user does not affect the user’s home directory or method and result files.

12.3.5 Defining new home directories

To define an existing directory as a home directory for a user, select the directory in the **Home directory** field in the **User setup** dialogue box. Any directory may be used as a home directory. As a recommendation in network installations, place the home directory on a drive which is addressed by the same drive letter from all computers in the network.

To create a new home directory at the same time as you create a user. Check the **Create home directory** box in the **New user** dialogue box and enter the directory name and drive in the appropriate fields.

(To create new directories under a home directory, use the **New directory** command in the Main menu (see Section 3.2). This operation does not require special authorization.)

12.3.6 Deleting home directories

To delete home directories, click on **Delete** in the **Access** panel of the **User setup** dialogue box. Select a directory to delete in the **Delete directory** dialogue box that appears and click on **OK**.

> *Important*
> It does not matter which directories are marked in the **Directories** list in the **User setup** dialogue box when you click on **Delete**. This list shows the directories to which the currently marked user has access, not the directories that will be deleted.

All methods, result files and subdirectories in a directory will be deleted when the directory is deleted.

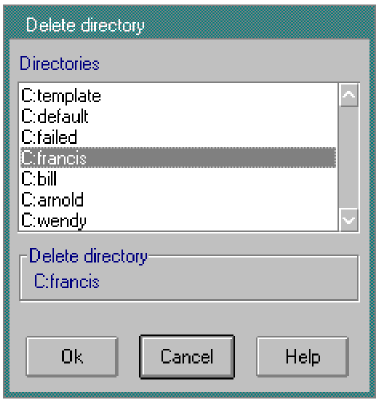


Figure 12-9. *Dialogue box for deleting home directories.*

You cannot delete

- ••a home directory to which a user is assigned. To delete such a directory, you must first either delete the user or change the home directory assignment for this user. Select the user in the **User setup** dialogue box and assign a different home directory.
- ••a directory to which several users share access. To delete such a directory, remove the access rights from each user first.

12.4 Audit trails

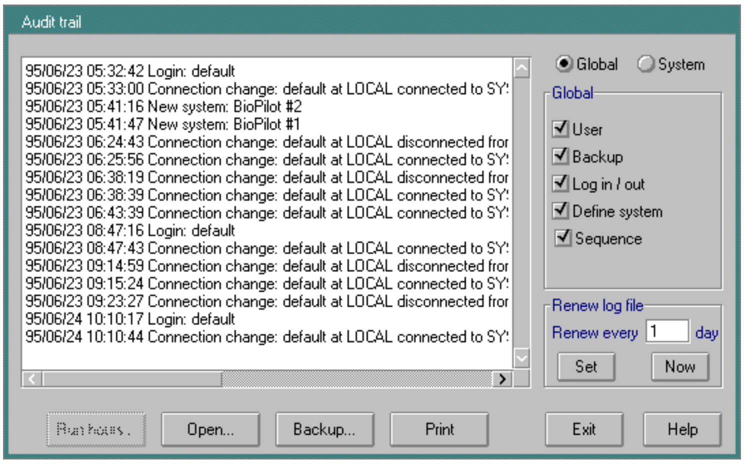
The audit trail, accessed under **Administration:Audit trail** in the Main menu, provides a full record of UNICORN OS usage and system activity for the system administrator. The audit trail may be viewed in global mode (all systems in the installation) or system mode (one chosen system).

12.4.1 Examining audit trails

In the audit trail window, choose whether you wish to view the global or system trail.

Global audit trails

Check the items you want to display in the **Global** panel. All items are recorded in the audit trail: the check boxes in the **Global** panel only control which items are displayed. Global audit trail files are saved on the server disk in a network installation, and a network connection is required to examine global audit trails.



```
95/06/23 05:32:42 Login: default
95/06/23 05:33:00 Connection change: default at LOCAL connected to SY!
95/06/23 05:41:16 New system: BioPilot #2
95/06/23 05:41:47 New system: BioPilot #1
95/06/23 06:24:43 Connection change: default at LOCAL disconnected fror
95/06/23 06:25:56 Connection change: default at LOCAL connected to SY!
95/06/23 06:38:19 Connection change: default at LOCAL disconnected fror
95/06/23 06:38:39 Connection change: default at LOCAL connected to SY!
95/06/23 06:43:39 Connection change: default at LOCAL connected to SY!
95/06/23 08:47:16 Login: default
95/06/23 08:47:43 Connection change: default at LOCAL connected to SY!
95/06/23 09:14:59 Connection change: default at LOCAL disconnected fror
95/06/23 09:15:24 Connection change: default at LOCAL connected to SY!
95/06/23 09:23:27 Connection change: default at LOCAL disconnected fror
95/06/24 10:10:17 Login: default
95/06/24 10:10:44 Connection change: default at LOCAL connected to SY!
```

● Global ○ System

Global
☑ User
☑ Backup
☑ Log in / out
☑ Define system
☑ Sequence

Renew log file
Renew every 1 day

Figure 12-10. *Global audit trails.*

User Displays all user creation, deletion and redefinition operations.

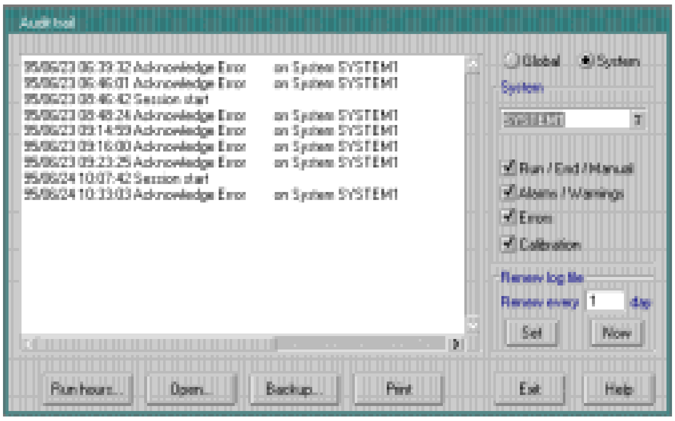
Backup Displays backup operations for global audit trail files.

Log in/out Displays all login and logout attempts with the name of the user logging in or out, including failed login attempts.

Define system Displays all system definition, deletion and redefinition events.

Sequence Displays sequence start operations.

System audit trails
Choose the system for which the audit trail is to be displayed and check the items you want to display in the **System** panel. All items are recorded in the audit trail: the check boxes in the **System** panel only control which items are displayed. System audit trail files are saved on the local station to which the system is physically connected, and may be examined from the local station without logging in to the network. System audit trail files can be viewed from any computer in a network installation.



```
95/06/23 06:39:32 Acknowledge Error     on System SYSTEM1
95/06/23 06:46:01 Acknowledge Error     on System SYSTEM1
95/06/23 08:46:42 Session start
95/06/23 08:48:24 Acknowledge Error     on System SYSTEM1
95/06/23 09:14:59 Acknowledge Error     on System SYSTEM1
95/06/23 09:16:00 Acknowledge Error     on System SYSTEM1
95/06/23 09:23:25 Acknowledge Error     on System SYSTEM1
95/06/24 10:07:42 Session start
95/06/24 10:33:03 Acknowledge Error     on System SYSTEM1
```

○ Global ● System

System
SYSTEM1

☑ Run / End / Manual
☑ Alarms / Warnings
☑ Errors
☑ Calibration

Renew log file
Renew every 1 day

Figure 12-11. System audit trails.

Run/End/Manual Displays times for run start and completion and for manual operation.

Alarms/Warnings Displays alarms and warnings for the system.

Errors Displays system errors.

Calibration Displays monitor calibration operations.

Viewing older audit trails
When you choose **Administration:Audit trail**, the audit trail window displays the current audit trail. To view an older audit trail, click on **Open** in the audit trail window and choose the file to open. The drive is automatically selected according to the type of audit trail file. Files are named by date and serial number. Choose **Current log file** to return to the current audit trail after viewing older files.

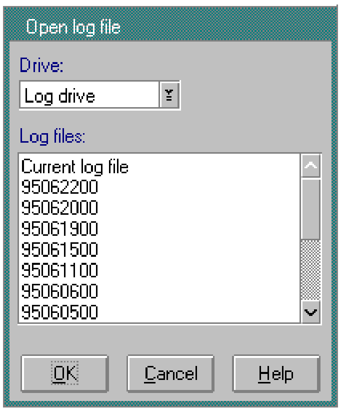


Figure 12-12. *Dialogue box for opening old audit trail files.*

Printing audit trails
Click on **Print** in the audit trail window menu prints the audit trail file as currently displayed in the window.

12.4.2 Renewing audit trail files

The audit trail file is renewed at regular intervals between 1 and 30 days. To set the interval, choose **Administration:Audit trail** from the Main menu. Enter the required interval in the **Renew every** field and click on **Set**. The new setting will take effect from the time the change is made e.g. if the setting is changed to 7 days at 10 a.m. on a Monday, the file will be renewed at 10 a.m. every Monday.

You can also start a new audit trail file at any time by clicking on **Now** in the **Renew log file** panel. This will not affect the automatic setting, e.g. if the audit trail is set to renew at 10 a.m. every Monday, and you click on **Now** on a Friday, a new file will be started immediately and another new file will be started on the following Monday morning.

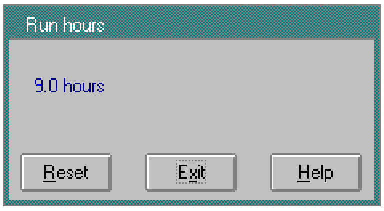
12.4.3 Backing up audit trail files

Click **Backup** in the audit trail window to make copies of audit trail files on diskette. Choose whether to copy or move the files to diskette and click on **Backup**. The **Move** alternative is recommended to save disk space. Backup operations are recorded in the audit trail.

Note: The **Backup** command simply copies the audit trail file to diskette. It does not use the OS/2 backup command.

12.4.4 System run hours

In the system audit trail, click on **Run hours** to display the accumulated run time for the system (i.e. the time the system has been in manual or run mode). To reset the run hours to zero, click on **Reset** in the dialogue box. The reset event is recorded in the audit trail for the respective system.



Run hours

9.0 hours

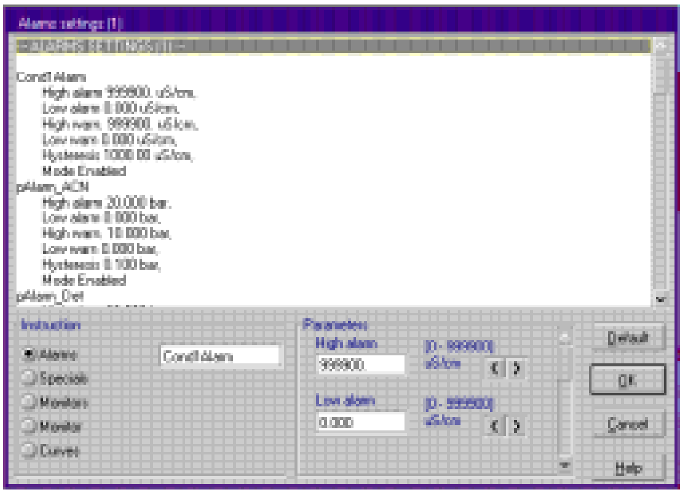
Figure 12-13. *Run hours in the system audit trail.*

13. System settings

The **System:Settings** command in the system control menu allows you to define global system settings for alarms and warnings and to choose which data will be stored in result files. Configuration access is required to change settings parameters (see Section 10.2). To access system settings, double-click on the System control desk-top icon for the appropriate system and choose **Settings** from the **System** menu.

Details of the settings for each system are defined in the system strategy. This section gives an overview of the principles of system settings.

The system settings should be established when the system is installed. Certain settings may need to be adjusted if system components are changed (e.g. alarm and warning limits) or for specific run purposes (e.g. monitor and curve settings). Some settings may also be set with an instruction in a method, overriding the global setting for the duration of the method. See Appendix B for settings defined in general synthesis strategies.



```
-- ALARMS SETTINGS (1) --

Cond1 Alarm
      High alarm 999900. uS/cm,
      Low alarm 0.000 uS/cm,
      High warn. 999900. uS/cm,
      Low warn 0.000 uS/cm,
      Hysteresis 1000.00 uS/cm,
      Mode Enabled
pAlarm_ACN
      High alarm 20.000 bar,
      Low alarm 0.000 bar,
      High warn. 10.000 bar,
      Low warn 0.000 bar,
      Hysteresis 0.100 bar,
      Mode Enabled
pAlarm_Det
```

Instruction: Cond1 Alarm

Parameters:
High alarm: 999900. [0 - 999900] uS/cm
Low alarm: 0.000 [0 - 999900] uS/cm

Figure 13-1. System settings in System control. The illustration shows the Alarms group of settings.

- ••To change settings, select a setting in the text panel and make the required changes in the parameters in the instruction box. The text panel will be updated when you select another setting.

- ••To restore a setting to its default setting as defined in the system strategy, select the setting in the text panel and click on **Default**.

When all required changes have been made, click on **OK**. Choosing **Cancel** will cancel all changes made since the dialogue box was last opened.

Important
Changes made to settings do not take effect until you click on **OK** to close the dialogue box.

Settings are divided into groups as listed in the instructions panel at the bottom of the dialogue box. Click on the appropriate group button or move to the first line in the respective group in the text window. You can also scroll between the groups in the text window.

13.1 Alarms

Alarms define upper and lower alarm and warning limits for process monitor signals:

- If the signal exceeds the ALARM limits, a buzzer sounds and an alarm message is displayed, and the process is paused (i.e. method execution is suspended and all pumps are stopped). The situation must be acknowledged and corrected before the process can be restarted.

- If the signal exceeds the WARNING limits, a warning message is issued without interrupting the process.

Alarm and warning messages are displayed on all stations with a connection to the system concerned, regardless of the activity currently being performed in UNICORN OS and regardless of the identity and access rights of the current user. Alarms and warnings can however only be acknowledged from the control mode connection.

If allowed by the system strategy, limits for certain monitor signals may also be set locally in a method, overriding the global setting as long as the method is in operation.

Error messages from the monitors and pumps are reported if the respective **Error** settings are **Enabled**.

Important
Alarms are not active unless the mode is set to **Enabled**. Use the scroll bar in the **Parameters** panel to access this setting if the mode parameter is not displayed.

The hysteresis setting for an alarm determines the extent to which the signal can oscillate around the warning limits without re-activating the warning (Figure 13-2).

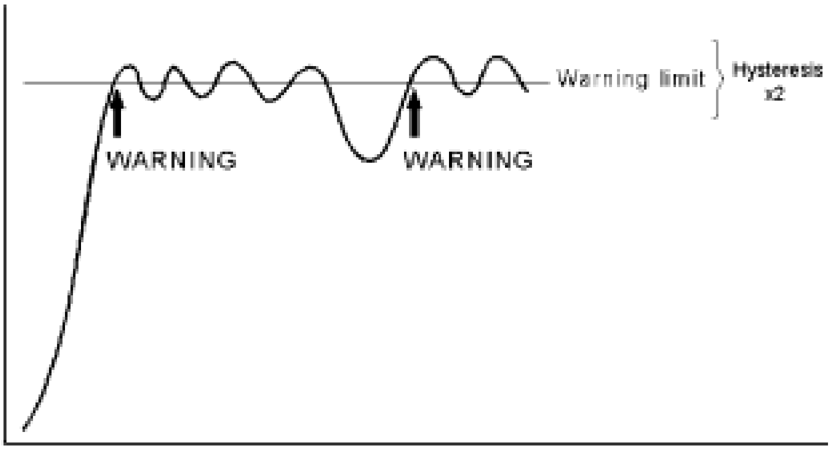
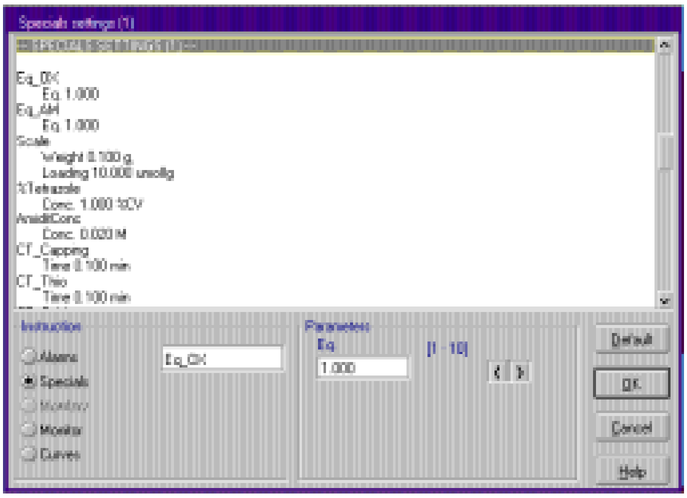


Figure 13-2. *The hysteresis setting defines the limits within which the signal may oscillate up or down from the threshold without re-activating a warning. After the signal has activated a warning, the warning will not be repeated as long as the signal remains within a window defined by the hysteresis setting above and below the warning limit. This prevents repeated warnings from noisy or oscillating signals close to the warning boundary.*
Hysteresis is only relevant for warnings, since an alarm puts the system in PAUSE at the first alarm.

Flow rate warning

If the software has calculated a flow rate that is more than the maximum capacity for a pump, a warning is displayed and the maximum flow rate will be used. This could mean a longer contact time than is set in the variables. this is mostly relevant for larger columns.

13.2 Specials



Specials settings (1)

```
Eq_OX
    Eq 1.000
Eq_AM
    Eq 1.000
Scale
    Weight 0.100 g,
    Loading 10.000 umol/g
%Tetrazole
    Conc. 1.000 %CV
AmiditConc
    Conc. 0.020 M
CT_Capping
    Time 0.100 min
CT_Thio
    Time 0.100 min
```

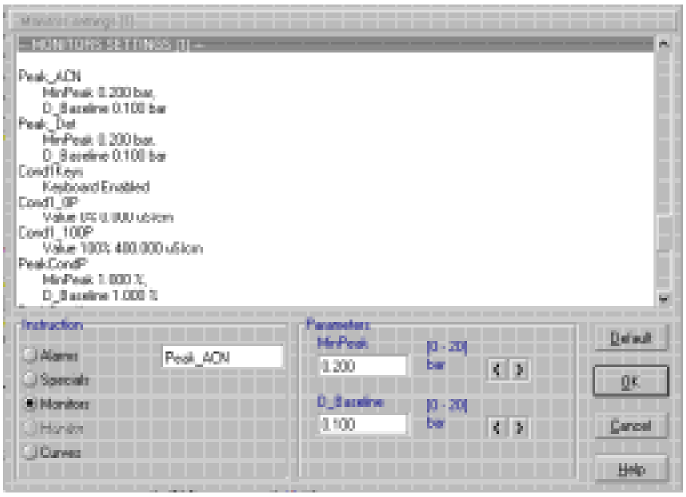
Instruction: ○ Alarms ● Specials ○ Monitors ○ Curves; Eq_OX

Parameters: Eq 1.000 [1 - 10]

Figure 13-3. Special settings in System control.

13.3 Monitors

Monitor settings determine how baseline and peaks in the monitor signal are handled when WATCH instructions are used (see Section 5.8.7), as well as a number of other settings for certain monitors such as whether the local keypad on the monitor can be used.



Monitor settings (1)

MONITORS SETTINGS (1)

```
Peak_ACN
    MinPeak 0.200 bar,
    D_Baseline 0.100 bar
Peak_Det
    MinPeak 0.200 bar,
    D_Baseline 0.100 bar
Cond1Keys
    Keyboard Enabled
Cond1_0P
    Value 0% 0.000 uS/cm
Cond1_100P
    Value 100% 400.000 uS/cm
PeakCondP
    MinPeak 1.000 %,
    D_Baseline 1.000 %
```

Instruction: ○ Alarms ○ Specials ● Monitors ○ Curves; Peak_ACN

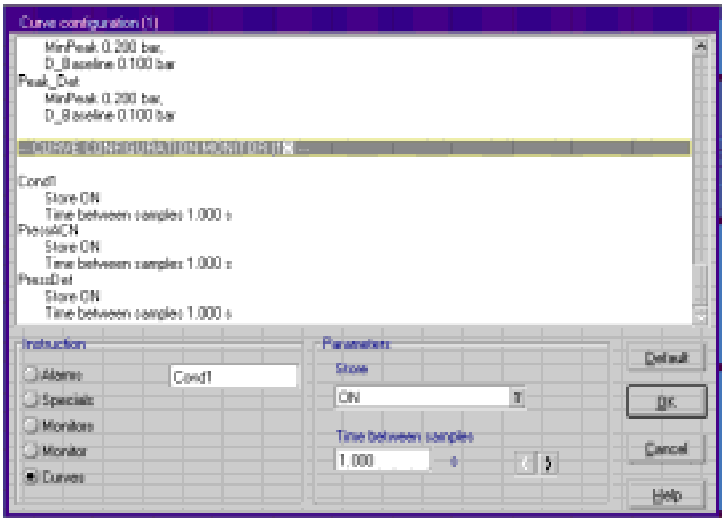
Parameters: MinPeak 0.200 [0 - 20] bar; D_Baseline 0.100 [0 - 20] bar

Figure 13-4. Monitor settings in System control.

13.4 Curves

Curve settings determine which monitor signals will be stored as curves in the result file. Check that **Store** is set to **ON** for all signals that are to be monitored.

> *Warning!*
> If a curve is set to **Store:OFF**, data from the monitor concerned cannot be displayed in the curves window during a process run, and will not be recorded in any way.



```
     MinPeak 0.200 bar,
     D_Baseline 0.100 bar
Peak_Det
     MinPeak 0.200 bar,
     D_Baseline 0.100 bar

-- CURVE CONFIGURATION MONITOR (1) --

Cond1
     Store ON
     Time between samples 1.000 s
PressACN
     Store ON
     Time between samples 1.000 s
PressDet
     Store ON
     Time between samples 1.000 s
```

Instruction: ○ Alarms ○ Specials ○ Monitors ○ Monitor ● Curves — Cond1

Store: ON

Time between samples: 1.000 s

Figure 13-5. *System settings in System control.*

Introductory material

Methods and Runs

Evaluation

System management

Appendices

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A. Technical specifications

A.1 System requirements

A.1.1 Hardware requirements[1]

Computer type:	IBM compatible PC, Compaq, with at least one free full-length expansion slot.
CPU:	Pentium/75 MHz or higher.
RAM minimum:	24 Mb.
Hard disc recomm.:	1 Gb
Floppy disc drive:	3.5” 1.44 Mb
Printer or plotter:	As supported by OS/2 Warp operating system.
Mouse:	Microsoft mouse or compatible.
Monitor:	Colour SVGA (Compaq Qvision)

The MCU for communication between UNICORN OS and the synthesis system(s) is supplied as part of the system(s) and is not included with UNICORN OS. The MCU is available for (E)ISA- and MCA-bus computers.

A.1.2 Software requirements

IBM Operating System OS/2[2] Warp version 3.0 or later.

A.1.3 Network requirements

The following are minimum network requirements for running UNICORN OS in a network installation:

- ••OS/2 version 3.0 or later.
- ••Etherlink III network card with 3C5x9.SYS driver (version dated 10th March 1995 or later).
- ••Novell NetWare[3] version 4.02 or later.
- ••A valid network connection.

[1] Hardware recommendations apply in February 1997. Contact your local sales office for details of the latest hardware recommendations.

[2] OS/2 is a registered trademark of IBM Inc.

[3] Novell and NetWare are registered trademarks of Novell Inc.

A.2 Control capacity

A.2.1 Stand-alone installations

Simultaneous control of up to four synthesis systems. Each module is separately configured in a system strategy supplied by Amersham Biosciences.

A.2.2 Network installations

Up to four synthesis systems may be connected to each local station in the network. Each station (local or remote) may control up to four systems simultaneously. Up to 20 computers with UNICORN OS installed may be logged in to the network at the same time. Each local station supports up to 8 simultaneous connections throughout the network (if more than one chromatography system is connected to a local station, the total number of connections to the systems installed on that system may not exceed 8, regardless of which station the connections are made from).

A.3 Data sampling

Data from synthesis system monitors is stored temporarily in data buffers in the local system controller. Data is transferred from the buffers to disk storage by UNICORN OS whenever a chromatogram is closed. Data is also saved to disk at preset intervals during a run, minimizing data loss in the event of communication failure.

The capacity of the data buffers is 16000 points for all. The initial sampling frequency for each monitor is set in the system strategy, and can be viewed and changed in the **Curves** group of **System:Settings**.

At an initial sampling frequency of 10 sample/s (10 Hz), the following resolutions apply for the curves:

Duration	Sampling frequency	No. of points	Resolution (s/point)
0-27 min	10 Hz	0-16000	0.1
27-53 min	5 Hz	8000-16000	0.2
53-107 min	2.5 Hz	8000-16000	0.4
107-203 min	1.25 Hz	8000-16000	0.8
...	...	...	...
...	...	...	...

To ensure maximum resolution for part of a run, issue a NEW_CHROMATOGRAM instruction at the beginning of the part. This empties the data buffers and resets the sampling frequency to that specified in the system strategy.

B. General strategy for Oligo Synthesis

This appendix alphabetically lists the instructions for methods, manual control, system settings and variables supported by the standard strategy for OligoPilot II. Most of the instructions apply to other synthesis systems, for example OligoProcess. The user is referred to the appropriate synthesis system manual for specific details.

B.1 Method instructions

B.1.1 Pump

Instruction	*Description*
CT_Coupl	Start flow with the reagent pump to create the contact time with the oxidation reagents The flow rate is determined by scale, **Eq_Amidite, %Tet, CV,** Delay volume
CapCT5_Flow	Start flow with the reagent pump to create the contact time with the oxidation reagents The flow rate is determined by **CV** and **CV_Cap**
OXCT5_Flow	Start flow with the reagent pump to create the contact time with the oxidation reagents The flow rate is determined by the **Scale** and **Eq_Ox**
ThioCT5_Flow	Start flow with the reagent pump to create the contact time with the oxidation reagents The flow rate is determined by **CV** and **CV_Thio**

CapCT6_Flow	Start flow with the Pump P6000 to create the contact time with the oxidation reagents The flow rate is determined by **CV** and **CV_Cap**
OXCT6_Flow	Start flow with the Pump P6000 to create the contact time with the oxidation reagents The flow rate is determined by the scale and **Eq_Ox**
ThioCT6_Flow	Start flow with the Pump P6000 to create the contact time with the oxidation reagents The flow rate is determined by **CV** and **CV_Thio**
Flow_ACN	Starts the flow with acetonitrile from Pump P6000 to the column or waste
Flow_Det	Starts the flow with detrit solution from Pump P6000 to the column or waste
Flow_Reag	Starts the flow with reagent, connected to valve x.x, to the column or waste
LFlow_ACN	Starts a linear flow of acetonitrile to the column or waste and is dependent on column diameter
LFlow_Det	Starts a linear flow of detrit solution to the column or waste and is dependent on column diameter
LFlow_Reag	Starts a linear flow of reagent to the column or waste and is dependent on column diameter
PFlow_ACN	Pressure-controlled flow of acetonitrile from Pump P6000 to the column or waste

PFlow_Det	Pressure-controlled flow of detrit solution from Pump P6000 to the column or waste
Vol_Amid	Set amidite and tetrazole with respect to the **Scale** (loading*weight), **%Tetrazole**, **AmiditeConc**, **Eq_AM**
Vol_Cap	Set capping with respect to **CV** and **CV_Cap**
Vol_Oxid	Set oxidation with respect to Scale (loading*weight), **Eq_Ox**, using the contact time flow rate
Vol_Thio	Set Thiolation with respect to **CV** and **CV_Thio**, using the contact time flow rate

B.1.2 Flowpath

Instruction	*Description*	*Comment*
Amidite:		Means: valve#pos#: port1pos#: port2pos1: valve5pos2: valve6pos1
ACN_A	to column 1.1: p1.0: p2.1 :5.2: 6.1	
A	to column 1.2: p1.0: p2.1 :5.2: 6.1	
ACN_C	to column 1.4: p1.0: p2.1 :5.2: 6.1	
C	to column 1.3: p1.0: p2.1 :5.2: 6.1	
ACN_G	to column 1.4: p1.0: p2.1 :5.2: 6.1	
G	to column 1.5: p1.0: p2.1 :5.2: 6.1	
ACN_T/U	to column 1.7: p1.0: p2.1 :5.2: 6.1	
T/U	to column 1.6: p1.0: p2.1 :5.2: 6.1	
ACN_A*	to column 2.1: p1.1: p2.1 :5.2: 6.1	
A*	to column 2.2: p1.1: p2.1 :5.2: 6.1	
ACN_C*	to column 2.4: p1.1: p2.1 :5.2: 6.1	
C*	to column 2.3: p1.1: p2.1 :5.2: 6.1	
ACN_G*	to column 2.4: p1.1: p2.1 :5.2: 6.1	
G*	to column 2.5: p1.1: p2.1 :5.2: 6.1	
ACN_T*/U*	to column 2.7: p1.1: p2.1 :5.2: 6.1	
T*/U*	to column 2.6: p1.1: p2.1 :5.2: 6.1	
ACN_X	to column 1.1: p1.0: p2.1 :5.2: 6.1	
X	to column 1.8: p1.0: p2.1 :5.2: 6.1	
ACN_Y	to column 2.1: p1.1: p2.1 :5.2: 6.1	
Y	to column 2.8: p1.1: p2.1 :5.2: 6.1	

Solvent:	
Beaucage	3.7: p1.0: 5.2: 6.1
ACN_3.1	to column 3.1: p1.0: 5.2: 6.1
Cap_A	to column 3.2: p1.0: 5.2: 6.1
Cap_B	to column 3.3: p1.0: 5.2: 6.1
ACN_3.4	to column 3.4: p1.0: 5.2: 6.1
OX	to column 3.5: p1.0: 5.2: 6.1
Extra_3.6	to column 3.6: p1.0: 5.2: 6.1
Tetrazole	to column 3.8: p1.0: 5.2: 6.1

Every amidite and solvent setting can also be set to waste. This means setting valve 6 from position 1 to position 2.

WasteOut:	
Waste_ACN	valve 6.1
Waste_Detrit	valve 6.2
Waste_A	valve 6.3
Waste_C	valve 6.4
Waste_G	valve 6.5
Waste_T	valve 6.6
Waste_X	valve 6.7
Waste_Y	valve 6.8
ACN Pump	to waste/to column
Detrit Pump	to waste/to column
Valve:	
1	1-8
2	1-8
3	1-8
4	1-8
5	1-3
6	1-3
Port:	
1	0-1
2	0-1
Recycle:	
ON	flowrate LFlow 0-1000 cm/h
OFF	flowrate LFlow 0-1000 cm/h

B.1.3 Alarms&Monitors

Instruction	*Description*	*Comment*
%Tetrazole	Percent tetrazole of the column volume	1-100%
AmiditeConc		0.01-0.5 M
Base_Id	Identification of the base that gives the trityl peak - used because Last eff. only considers bases with the same ID.	
ColDiam	Sets the column diameter in mm - used in Lflow functions	1-100 mm
Cond1Alarm	Sets the alarm and warning limits for the signal from the monitor	
CV	Sets the column volume for calculation of CT flows and volume of Capping and Beaucage	1-200
CV_Cap	Column volume of capping, ½ CapA and ½ CapB	0.1-10 CV
CV_Thiolat	Sets the column volume for thiolation	0.1-1.0 CV
Cycle_Start	Marks the start point for an integration and thus where from retention is calculated	
DelayVol	The dead volume from port1 to the column inlet	0.1-10 ml
Eq_AM	Equivalents of amidites, dependent on the scale	1-10 eq
Eq_OX	Equivalents of oxidation, dependent on the scale	1-10 eq
pAlarm_Det **pAlarm_ACN**	Sets the alarm and warning limits for the pressure on the Det and ACN pumps. An alarm will set the system in Pause. A warning will issue a warning message with the system in Run.	

*****CycleStart*****	Indicates the synthesis cycle start in order to calculate retention, and used by **Pause_at_Cycle_End** to Pause a synthesis	
PeakCond1	Sets the minimum peak (MinPeak) *Not* to be regarded as a disturbance when using the instruction Watch, and the limits (±D_Baseline) used by the instruction **Watch Stable_baseline**	
PumpError	Enables\disables the alarms from the pumps	
Scale	Loading (µmol/g) * weight (g)	10-250 µmol/g and 0.1-100 g respectively
Integrate_Start	Indicates when the method can start looking for **Int_Start_Level** to begin integration of the trityl peak.	
CT_Thio	Sets the contact time of Beaucage	
CT_Oxidate	Sets the contact time of oxidation	
CT_Capping	Sets the contact time of capping	

B.1.4 Watch

Instruction	*Description*	*Comment*
Watch_PressDet	Watch on Pump P6000 for detritylation	0-20 bar
Watch_PressACN	Watch on Pump P6000 for detritylation	0-20 bar
Watch_Cond1	Watch on conductivity monitor	
Watch_IntStatus	Watch on integration	ON/OFF
Watch_Off	Cancels a watch on a specified monitor.	
Watch_Efficiency		-1.0-151%

B.1.5 Other

Instruction	*Description*	*Comment*
Base	Defines the base for a block for calculating breakpoints. Each block must have a defined base.	Volume Time Column volume
Call	Calls a block unconditionally.	Block name
Continue	Resumes execution of a paused or held method. This instruction has the same effect as clicking on the *Cont.* button in System control.	–
End_Method	Terminates method execution, equivalent to clicking on the **End** button in System control.	–
Evaluate	Calls an evaluation procedure. The procedure must be stored together with the current method.	Procedure name
Hold	Places the system in Hold state. This instruction has the same effect as clicking on the **Hold** button in System control.	–
Loop	Runs the instructions between a start and a number of loops	No of loops (1 – 9999)
Loop_end	Marks the end of a loop.	–
Message	Generates a user-defined message which is recorded in the log book and may be displayed on the screen.	"Message" Mode: Screen / Noscreen
New_ chromatogram	Opens a new chromatogram icon in the result file. All data collected after the instruction will be stored under the new icon until another **New_chromatogram** instruction is issued.	Chromatogram name
Pause	Places the system in the Pause state for the specified length of time.	Time (-1 (infinite) – 9999.9 in minutes)
Ready	Indicates that the next step in a process sequence may start.	–

End_Block	Terminates a block and returns control to the point from which the block was called.	–
;	Inserts a comment in the method below the marked instruction.	Comment text

B.2 Manual control instructions

B.2.1 Pump

This group contains the same instructions as the Pump group in method instructions (Section B.1.1).

B.2.2 Flowpath

This group contains the same instructions as the Flowpath group in method instructions (Section B.1.2) with the addition of the following instruction:

B.2.3 Alarms&Monitors

This group contains the same instructions as the Alarms&Mon group in method instructions (Section B.1.3), in addition to the following:

Instruction	*Description*	*Parameters*
Efficiency_ Threshold	Can be used to set the watch on efficiency from off to on.	-
Pause_at_ Cycle_End	This sets the system to Pause at the next cycle start. Active only one time.	-

B.2.4 Other

Instruction	*Description*	*Parameters*
Call	Calls a block	Block name
Next_ breakpoint	Jump to the next breakpoint in the current method (only relevant when a method or block is running).	-
Record_on	Begins recording a run that has been started manually. A result file will be generated.	-

B.3 System settings instructions

B.3.1 Alarms Settings

Instruction	*Description*	*Comments*
Cond1Error	Enables/disables all alarms	Enabled/Disabled
Cond1Alarm	Sets the alarm and warning limits for the signal from the respective monitor	High alarm 0-99900 µS/cm Low alarm 0-99900 µS/cm High warn 0-99900 µS/cm Low warn 0-99900 µS/cm Hysteresis 0-99900 µS/cm Enabled/Disabled
Efficiency_ Threshold	Sets the threshold for an acceptable coupling efficiency. An efficiency below this threshold will pause the synthesis	Threshold 0-100% Enabled/Disabled
pAlarm_Det **pAlarm_ACN**	Sets the alarm and warning limits for the pressure on the Det and ACN pumps. An alarm will set the system in Pause. A warning will issue a warning message with the system in Run.	High alarm 0-20 bar Low alarm 0-20 bar High warn 0-20 bar Low warn 0-20 bar Hysteresis 0-5 bar Enabled/Disabled

B.3.2 Specials Settings

Instruction	*Description*	*Parameters*
%Tetrazole	Percent tetrazole of the column volume	Conc 1-100% CV
AmiditeConc		Conc 0.01-0.5 M
ColDiam	Sets the column diameter in mm - used in Lflow functions	Diameter 10-100 mm
CV	Sets the column volume for calculation of CT flows and volume of Capping and Beaucage	Volume 1-200 ml

CV_Cap	Column volume of capping, ½ CapA and ½ CapB	0.1-10 CV
CV_Thiolat	Sets the column volume for thiolation	Volume 0.1-10 CV
DelayVol	The dead volume from port1 to the column inlet	Volume 0.1-10 ml
Eq_AM	Concentration of amidite	Eq 0.1-10
Eq_OX	Equivalents of oxidation, dependent on the scale	Eq 1-10
Int_Values	Sets the level where an integration starts and ends	StartLevel ø=2000 µS/cm StopLevel ø=2000 µS/cm
P50Gain	A scaling factor to calibrate the flow of the P50 pump	Gain 0.75-1.25
PumpError	Enables\disables the alarms from the pumps	ACN, Detrit and Reagent, each Enabled/Disabled
Scale	Loading (mol/g) * weight (g)	Weight 0.1-1000 g Loading 10-250 µmol/g
CT_Thio	Sets the contact time for Beaucage	0.1-10 min
CT_Oxidate	Sets the contact time for Oxidate	0.1-10 min
CT_Capping	Sets the contact time for Capping	0.1-10 min

B.3.3 Monitors Settings

Instruction	*Description*	*Parameters*
Cond1Keys		Keyboard Enabled/Displayed
Peak_Det **Peak_ACN**	Sets the alarm and warning limits for the pressure on the Det and ACN pumps. An alarm will set the system in Pause. A warning will issue a warning message with the system in Run.	MinPeak 0-20 bar D_Baseline 0-20 bar

PeakCond1	Sets the minimum peak (MinPeak) *Not* to be regarded as a disturbance when using the instruction Watch, and the limits (±D_Baseline) used by the instruction **Watch Stable_baseline**	MinPeak 0-99900 µS/cm D_Baseline 0-99900 µS/cm

B.3.4 Curve Configuration

Instruction	*Description*	*Comment*
Cond1	Set the specific signal curve on/off	ON / OFF
PressDet	for storing in the Result file. The	
Press ACN	time between samples determines	Time between
	the frequency with which curve data	samples
	is recorded	0->1.000 s

B.3.5 Method variables

(in alphabetical order)

Variable	*Units*	*Description*
Col_Diam	mm	Column diameter for linear flow rates
Column_Volume	ml	Column volume for text method
Conc_of_DNA_ amidite	M	Sets the DNA amidite concentration
Conc_of_RNA_ amidite	M	Sets the RNA amidite concentration
CT_Oxidation_DNA	min	Sets the oxidation contact time
CT_Oxidation_RNA	min	Sets the oxidation contact time
CT_Thio	min	Sets the Beaucage reagent (thiolation) contact time
CT_Capping	min	Sets the capping reagent contact time
CV	ml	Column volume for calculation of functions in the strategy
CV_CT_Capping	CV	Sets the number of column volumes that the CT_Capping will last

CV_CT_Ox_DNA	CV	Sets the number of column volumes that the oxidation will last
CV_CT_Ox_RNA	CV	Sets the number of column volumes that the oxidation will last
CV_Capping	CV	Sets the number of column volumes of capping reagent
CV_Capping_wash	CV	Sets the number of column washes after Capping contact flow
CV_Column_wash	CV	Number of column volumes used in column wash block
CV_Coupling_wash	CV	Number of column volumes of wash after coupling
CV_detrit_wash	CV	Number of column volumes of detrit wash
CV_tetrazole_DNA	%	Sets the % of the column volume for tetrazole in DNA cycles
CV_tetrazole_RNA	%	Sets the % of the column volume for tetrazole in RNA cycles
CV_Thio	CV	Sets the number of column volume after of Beaucage reagent
Detrit_flow	cm/h	The linear flow used in detritylation block
Detrit_Pressure_Flow	bar	Maximum flow pressure of detrit
Efficiency_threshold	%	Minimum acceptable threshold efficiency
Eq_oxidation_DNA	eq	Number of equivalents of oxidation
Eq_oxidation_RNA	eq	Number of equivalents of oxidation
Eq_DNA_amidite	Eq	Number of equivalents of DNA amidite
Eq_RNA_amidite	Eq	Number of equivalents of RNA amidite
Loading_of_support	µmol/g	To calculate the scale
Recycle_Time	min	Sets the time of recycling of amidite and tetrazole
Weight_of_support	g	To calculate the Scale

C. Evaluation functions and instructions

This appendix describes the functions implemented in the Evaluation module. There are four sections in the appendix:.

C.1 describes how the smoothing functions are calculated

C.2 gives an basic introduction into baseline calculation theory which is an essential part of peak integration

C.3 describes the peak table column components

C.4 the Procedure Editor instruction types are described which are used to build up an evaluation procedure

C.1 Smoothing algorithms

C.1.1 Moving Average

For each data point in the source curve, the processed target curve is calculated as the average of the data points within a window centred on the source data point. The width of the window is determined by the parameter value, expressed as number of data points.

When the source point is less than half the window size from the beginning or the end of the curve, the average is calculated symmetrically round the source point over as many data points as possible.

Increasing the window width increases the smoothing effect.

The filter algorithm only accepts odd integer parameter values between 1 and 51. If an even number has been given it is incremented by one.

C.1.2 Autoregressive

The first data point in the source curve is copied to the processed target curve. For each subsequent data point, the previous processed target point is multiplied with the parameter value and added to the current source data point. The result is then divided by the parameter value plus 1 according to the following formulae:

$$t_1 = s_1$$

$$t_n = \frac{(p*t_{n-1} + s_n)}{(p+1)}$$

where

t_n = current processed target point

t_{n-1} = previous processed target point

s_n = current source point

p = smoothing parameter value

Increasing the parameter value increases the smoothing effect. The filter algorithm accepts integer parameter values between 1 and 10.

C.1.3 Median

For each data point in the source curve, the processed target curve is calculated as the median of the data points within a window centred on the source data point. The width of the window is determined by the parameter value, expressed as number of data points.

When the source point is less than half the window size from the beginning or the end of the curve, the median is calculated symmetrically round the source point over as many data points as possible.

Increasing the window width increases the smoothing effect. To completely remove a noise spike, the window width should in principle be slightly more than twice the width of the spike.

The filter algorithm only accepts odd integer parameter values between 1 and 51. If an even number has been given it is incremented by one.

C.2 Baseline calculation theory

The baseline calculation can schematically be described in three steps:

1. Find baseline segments

2. Select baseline points

3. Draw the baseline.

C.2.1 Finding baseline segments

In the first step, four baseline parameters are used to find the baseline segments. The parameters can be seen in the **Integrate:Calculate baseline** function or by pressing the **Baseline settings** command button in the **Integrate:Peak integrate** function. The default values for the parameters are determined from the source curve.

The baseline segments are found by searching for all parts of the source curve which

1. are longer than the **Shortest baseline segment**.
   This parameter determines the minimum length for a part of the source curve to be considered a possible baseline segment.

2. have no point outside the **Noise window**.
   The noise window is defined as a rectangular corridor parallel to the slope of the curve and centred on the first and last points within the currently inspected segment.

3. slope less than the **Slope limit**.
   This limits the maximum slope of the baseline to differentiate baseline segments from peaks.

4. are lower than the **Max baseline level**.
   Determines the highest acceptable signal level for the baseline. This parameter is by default set to have no influence on the baseline calculation and is seldom necessary to adjust.

The parameters can be illustrated as a rectangular box in which the source curve has to fit to be identified as a baseline segment, see Figure C-1. The length of the box corresponds to the **Shortest baseline segment** and the height of the box corresponds to the maximum level of noise on the baseline segments and is referred to as the **Noise window**.

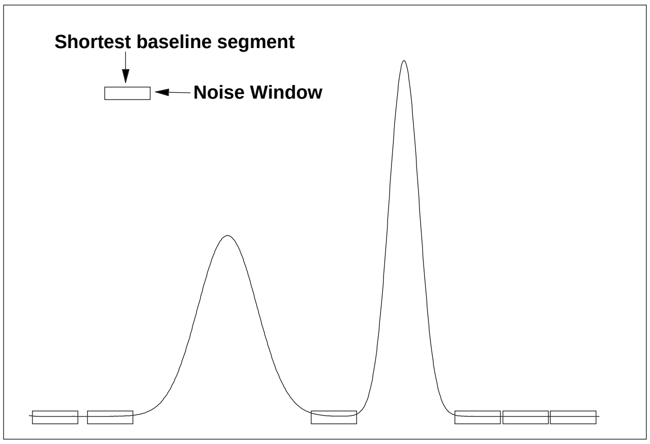


Figure C-1. *Baseline box with Shortest baseline segment and Noise window.*

The rectangular box is allowed to be tilted with a maximum slope corresponding to the **Slope limit**, see Figure C-2. The box is not allowed to move up above the **Max baseline level**.

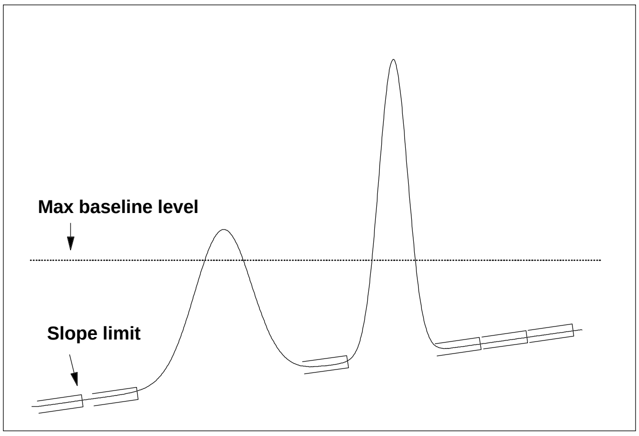


Figure C-2. *Slope limit and Max baseline level.*

When looking for baseline segments, the box is virtually moved along the source curve in steps of 1/3 of the **Shortest baseline segment**. A baseline segment is found whenever the currently examined part of the source curve fits completely within the box.

The found baseline segments are joined by connecting adjacent segments, provided that the slope of the joining lines does not exceed the **Slope limit**.

C.2.2 Selecting baseline points

In the second step, the baseline segments are replaced by a large number of baseline points. A baseline point is placed at the start and end of each segment. The line between these will also be filled with points. The baseline points are shown as pale blue crosses in the **Integrate:Edit baseline** function.

C.2.3 Drawing the baseline

The baseline points are used to create the baseline curve using a spline interpolation. The spline function ensures that the baseline curve is guided by the baseline points, but the curve does not necessarily pass through them. The baseline will thus be a smoothly curved function passing close to or through the points. To reduce the effect of noise on the peak integration, the created baseline is adjusted by forcing it equal to the source curve in every position where the difference between the baseline and the source curve is small enough. If the **Accept negative peaks** option is off, the baseline will be forced down to the level of the source curve whenever the created baseline goes above the source curve.

C.2.4 Estimating the baseline parameters from the source curve

The baseline parameters can sometimes be difficult to set. Rough estimates can be found by analyzing the source curve.

Measuring the Shortest baseline segment using curve co-ordinates
If you are uncertain of the length of the **Shortest baseline segment**, you can try to measure it directly on your chromatogram. Locate the shortest segment of the curve that you consider as a part of the baseline and measure the length of the segment using the **XY** icon on the chromatogram. Insert this value as the **Shortest baseline segment** value.

Measuring the noise level using curve co-ordinates
As for measuring the **Shortest baseline segment**, curve co-ordinates can be used in exactly the same manner to measure noise levels on the source curve. First use the **Zoom** function to select a part of the curve representative of the baseline noise. Measure the Y-axis co-ordinates, using the appropriately selected Y-axis scale, to calculate the noise range as the difference between the max and min values. Add an extra 20% and insert this value as the **Noise window** value.

Measuring the Slope limit using Differentiate and curve co-ordinates
To measure the slope at any point on the curve:

1. Select **Operations:Differentiate**. A box will appear.

2. Select the desired source curve, check that a **First order** calculation is selected and click on **OK**. The differentiated curve will appear in the active chromatogram.

3. Measure the Y-axis values on the differentiated curve using the **XY** curve co-ordinates function. Remember to select the appropriate Y-axis scale. Any Y-axis value is interpreted as the UV curve slope at the selected retention point.
   If the differentiated curve is very noisy, it can be filtered using a light Moving average filter in the **Operations:Smooth** function.

4. Determine the highest slope value of the baseline (non-peak) part of the curve. Add an extra 10% and insert this value as the **Slope limit**.

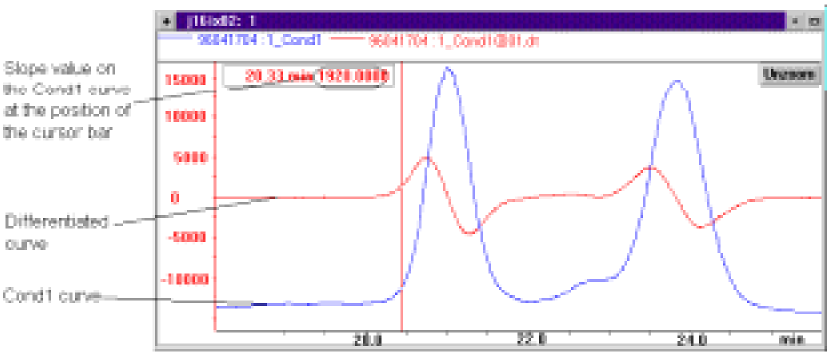


__Figure C-3.__ Measurement of the Slope limit after differentiation.

C.3 Peak table column components

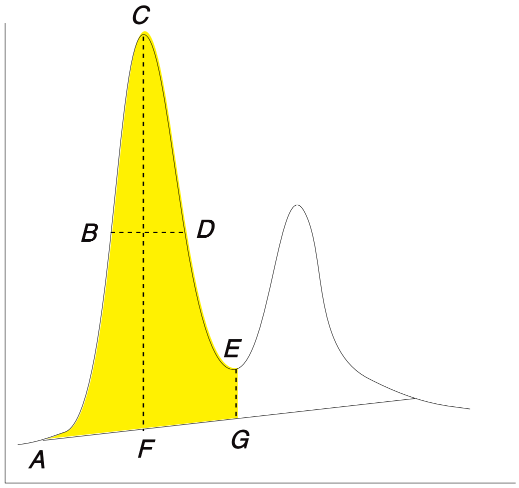


Figure C-4. *Diagram illustrating peak parameters. See the parameter list below for explanations.*

Peak name	Name of peak.
Retention	(time or volume base) Retention at the peak maximum (C in Figure C-4).
Width	(time or volume base) Difference in retention between the peak end and peak start (G-A in Figure C-4).
Area	(time or volume base) Calculated as the area between the curve and baseline, between the peak start and peak end (shaded in Figure C-4).
Height	Maximum amplitude above the baseline (C-F in Figure C-4).
Peak endpoint retention	(time or volume base) Retention value at peak start and peak end (A, G in Figure C-4).
Width at half height	(time or volume base) Calculated by taking the maximum height of the peak above the baseline, then determining the peak width at half this value above baseline (D-B in Figure C-4, where BD bisects CF).
Percent of total area	(time or volume base) Peak area as a percent of the total area under the curve above the baseline. Note that this value may differ in time and volume base if the flow rate is not constant throughout the method.
Percent of total peak area	(time or volume base) Peak area as a percent of the sum of all integrated peaks. Note that this value may differ in time and volume base if the flow rate is not constant throughout the method.

Type of peak limits	Identifies the criteria for peak start and peak end as either the baseline intersection or drop-line to the baseline.
Peak endpoint heights	Amplitude above the baseline at left (A in Figure C-4) and right peak limits (E-G in Figure C-4).
Fraction tube id	Fraction number at peak start, peak maximum and peak end.
Baseline height	Baseline amplitude at peak start, peak maximum and peak end (A, F and G in Figure C-4).
Sigma	Standard deviation for a Gaussian-shaped peak. For definition see below*
Resolution	Peak resolution. For definition, see below**.
Plate height (HETP)	Height equivalent to theoretical plate and plates/metre. The column height must be entered in the **Integrate** dialogue box for this parameter to be calculated. For definition, see below‡.
Asymmetry	Peak asymmetry (indicator of column packing). For definition, see below†.
Peak name	Name of the peak.

**Sigma*

$$\mathrm{Sigma} = \frac{\sum \left(Y_i \left(i - i_{max} \right)^2 \right)}{A_{peak}}$$

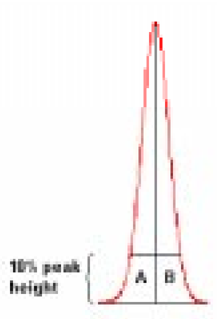
where:
Y_i is the amplitude at retention sample number i on the peak
i_{max} is the sample number of the peak maximum
A_{peak} is the peak area.

The peak width for a Gaussian peak is (4 × Sigma).

***Resolution*

$$R_s = \frac{\left(V_{R2} - V_{R1} \right) \times 1.177}{w_{h1} + w_{h2}}$$

where:
V_{R1} = elution volume for peak 1
V_{R2} = elution volume for peak 2
w_{h1} = peak width at half height for peak 1
w_{h2} = peak width at half height for peak 2.



[†]*Asymmetry*

Asymmetry = width B / width A, where A and B are measured at 10% of the peak height.

[‡]*HETP*

$HETP = L/N$

$N = 5.54 * (V_R/w_h)^2$

where

N = no. of theoretical plates

L = bed height in cm

V_R = peak elution volume or time

w_h = peak width at half height expressed in the same units as V_R

C.4 Evaluation procedure instructions

C.4.1 Curve operations

Instruction	*Description*	*Parameters*
ADD	Adds two curves to gain a third curve which is the sum of the two curves. The two curve sources must have the same y-axis unit and not be fraction or injection curves or else a run time error will occur.	First curve source Second curve source Resulting curve
AMP_MUL	Multiplies the amplitude of the curve in Curve source by the multiplication factor and stores the result in the Resulting curve .	Curve source Resulting curve Multiplication factor
AMP_SHIFT	Shifts the amplitude of the curve in Curve source by the shift factor and stores the result in the Resulting curve.	Curve source Resulting curve Shift factor
CLEAR	Clears specified curve from the working memory of the computer.	Curve source
COPY	Copies the curve in Curve source to Resulting curve.	Curve source Resulting curve
CUT	Cuts out the part of the curve in Curve source between Left and Right Limits and stores the result in the Resulting curve.	Curve source Resulting curve Left limit Right limit
DERIVATE	Differentiates the curve in Curve source (first or second order) and stores the result in Resulting curve. The y-axis of the Resulting curve will be a normalized scale without unit.	Curve source Resulting curve First Order or Second Order
DIV	Divides two curves to gain a third curve which is the quotient of the two curves. The two curve sources must have the same y-axis unit, or one of them must have AU-scale and the other Activity-scale, and not be fraction or injection curves. The y-axis of the Resulting curve will be a normalized scale without unit.	First curve source Second curve source Resulting curve
HISTOGRAM	Creates a histogram from any non-fraction curve (Curve source 1) and a fraction curve (Curve source 2_frac), and stores the result in the Resulting curve. If Curve source 2 is not a fraction curve a run time error will occur. The y-axis of the Resulting curve will be the same as that of the first curve source.	First curve source Second curve source Resulting curve

INTEGRATE	Performs a mathematical integration of the _frac curve in the Curve source and stores the result in Result curve. This instruction is not the same as **Peak integrate** which performs a real peak integration.	Curve source Resulting curve
POOL_ FRACTIONS	Pools fractions from the curve in Curve source and stores the result in the Resulting curve. The fractions are pooled from the first selected fraction to the last selected fraction. If Curve source is not a fraction curve, or First or Last is not an existing identification, a run time error will occur.	Curve source Resulting curve First fraction to pool Last fraction to pool
RET_MUL	Multiplies the retention of the curve in Curve source by the Multiplication factor and stores the result in the Resulting curve.	Curve source Resulting curve Multiplication factor
RET_SHIFT	Shifts the retention of the curve in Curve source by the Shift factor and stores the result in the Resulting curve.	Curve source Resulting curve Shift factor
SMOOTH_AR	Smooths Curve source with an autoregressive filter and stores the result in Resulting curve. The Filter parameter decides the strength of the filter.	Curve source Resulting curve Filter
SMOOTH_ MA	Smooths the curve in Curve source with a moving average filter and stores the result in Resulting Curve. The Filter width parameter decides how many samples wide the filter is.	Curve source Resulting curve Filter width
SMOOTH_ MEDIAN	Smooths the curve in Curve source with a median filter and stores the result in Resulting curve. The Filter width parameter decides how many samples wide the filter is.	Curve source Resulting curve Filter width
SUB	Subtracts two curves to gain a third curve which is the difference of the two curves. The two curve sources must have the same y-axis unit and not be fraction or injection curves.	First curve source Second curve source Resulting curve
T_DIV	Divides two curves to gain a third curve which is the quotient of the two curves. The two curve sources must have the same y-axis unit, or one of them must have the AU scale and the other the Activity scale, and not be a fraction or injection curves. The threshold values are used to avoid division of numbers close to zero. At those points where Curve source 1 has amplitude less than Threshold1, or Curve source 2 has amplitude less than Threshold2, the result of the division is defined to be 1.0. The y-axis of the curve will be the same as that of the first curve source.	First curve source Second curve source Resulting curve Threshold1 Threshold2

C.4.2 Integration

Instruction	*Description*	*Parameters*
CALCULATE_ BASELINE	Calculates a baseline from the curve in Curve source. The baseline is stored in the Resulting curve. DEFAULT can be selected in the Baseline parameters which will than calculate default baseline parameters for each new curve.	Curve source Resulting curve Noise Window Shortest baseline segment Slope limit Max baseline level
CLEAR_ PEAKTABLE	Clears the peak table in Peak table source from the working memory of the computer.	Peak table source
COPY_PEAK TABLE	Copies a peak table from Peak table source to Resulting peak table.	Peak table source Resulting peak table
NEGATIVE_ PEAKS	Controls the baseline behaviour in subsequent baseline calculations. If OnOff is *ON* then the baseline may be drawn above the curve and negative peaks may be detected by PEAK_INTEGRATE. If OnOff is *OFF* then the baseline is never drawn above the curve.	OnOff
PEAK_ INTEGRATE	Performs a peak integration on the curve in Curve source and stores the resulting peak table in Resulting peak table. It is assumed that the baseline is subtracted.	Curve source Resulting peak table
PEAK_ WINDOW	Specifies which part of the curve in Curve source that will be integrated. Peaks between retention Left limit and Right limit will be detected if the OnOff parameter is set to On. If OnOff is set to Off, the whole curve will be used for integration.	Curve source Left limit Right limit OnOff
REJECT_ PEAKS	Specifies which peaks that are interesting to include in a peak table when peak integrating. If Area Less Than has a numerical value (not *OFF*) then only peaks larger than this area will be included in the peak table. If Height Less Than is specified then no peaks lower than this height are included. Similarly if the Width Less Than parameter is not *OFF* then only peaks wider than this value are accepted and if Width More Than is not *OFF* peaks narrower than this value is accepted. Finally if Peak Must be One of xx Largest is specified then at most so many peaks as specified will be included in the peak table and if more than number of peaks are detected the ones with the largest area are selected. Any combination of conditions is allowed. If all parameters are *OFF* then every detected peak are included in the peak table.	Height less than Width less than Width more than Area less than Peak must be one of xx largest

SET_ COLUMN_ HEIGHT	Sets the column height for the peak integration calculation of the HETP value. The Column height parameter is the height of the column in centimetres. If Column height is *OFF* then the HETP value is not calculated for the following integrations.	Column Height

C.4.3 File Operations

CURVE_ OPEN	Opens the curve specified in the Result file defined in File name and stores it in Resulting curve. If "*" is entered as File name the current result file will be used. The File name parameter may include a path from the users root directory.	File name Curve name Resulting curve
IMPORT_ CURVE	Imports a curve to the current chromatogram from another chromatogram (in the current file) and stores it in the Resulting curve.	Chromatogram name Curve source Resulting curve
IMPORT_ PEAKTABLE	Imports a peak table to the current chromatogram from another chromatogram (in the current file) and stores it in the Resulting curve.	Chromatogram name Peak table source Resulting peak table
PEAKTABLE_ OPEN	Opens the specified Peak table in the Result file defined in File name and stores it in the Resulting peak table. If "*" is entered as File name the current Result file will be used. The File name parameter may include a path from the current users root directory.	File name Peak table name Resulting peak table

C.4.4 Export

Instruction	*Description*	*Parameters*
EXPORT_ CURVE_ ASCII	Exports the curve in Curve source to the file defined in Export to File in ASCII format. In the part of Curve source limited by Left limit and Right limit Every <n> samples are exported.	Curve source Left limit Right limit Every <n> sample Export to file
EXPORT_ CURVE_WKS	Exports the curve in Curve source to the file defined in Export to File in WKS format. In the part of Curve source limited by Left limit and Right limit Every <n> samples are exported.	Curve source Left limit Right limit Every <n> sample Export to file

EXPORT_ DOC_ASCII	Exports the documentation in the current result file in ASCII format to the file defined in Export to file. If all parameters to this function is *OFF* then no documentation is exported. If at least one of them is *ON* then the documentation will be exported and the corresponding parts will be included in the exported file.	ONOFF Variables ONOFF Scouting ONOFF Start Protocol ONOFF Questions ONOFF RefCurves ONOFF EvalProc ONOFF Method Info ONOFF Method Notes ONOFF StartNotes ONOFF RunNotes ONOFF EvalNotes ONOFF Sys Settings ONOFF Calibration ONOFF LogBook ONOFF Result Name ONOFF Column Parameters NAME Export to file
EXPORT_ DOC_WKS	Exports the documentation in the current result file in WKS format to the file defined in Export to file. If all parameters to this function is *OFF* then no documentation is exported. If at least one of them is *ON* then the documentation will be exported and the corresponding parts will be included in the exported file.	ONOFF Variables ONOFF Scouting ONOFF Start Protocol ONOFF Questions ONOFF RefCurves ONOFF EvalProc ONOFF Method Info ONOFF Method Notes ONOFF StartNotes ONOFF RunNotes ONOFF EvalNotes ONOFF Sys Settings ONOFF Calibra tion ONOFF LogBook ONOFF Result Name ONOFF Column Parameters NAME Export to file
EXPORT_ EVAL_LOG_ ASCII	Exports an evaluation log in ASCII format to the file defined in Export to file.	Export to file
EXPORT_ EVAL_LOG_ WKS	Exports an evaluation log in WKS format to the file defined in Export to file.	Export to file
EXPORT_ METHOD_ ASCII	Exports a method to the file defined in Export to file in ASCII format. If all parameters are *OFF* then no method is exported. If Main is *ON* then the main method is included and if Blocks is *ON* then all blocks are included in the exported file.	Main Blocks Export to file

EXPORT_ METHOD_ WKS	Exports a method to the file defined in Export to file in WKS format. If all parameters are *OFF* then no method is exported. If Main is *ON* then the main method is included and if Blocks is *ON* then all blocks are included in the exported file.	Main Blocks Export to file
EXPORT_ PEAKTABLE_ ASCII	Exports the peak table in Peak table source to the file defined in Export to file in ASCII format.	Peak table source Export to file
EXPORT_ PEAKTABLE_ WKS	Exports the peak table in Peak table source to the file defined in Export to file in WKS format.	Peak table source Export to file

C.4.5 Chromatogram functions

Instruction	*Description*	*Parameters*
COPY_ CHROM	Creates a copy of the specified chromatogram. If "*" is used as source then the current (default) chromatogram is used. If "*" is used as destination then a default name will be created for the copy.	From chromatogram name To chromatogram name
CREATE_ NEW_ CHROM	Creates a new chromatogram with the given name. If "*" is used for the chromatogram name a default name will be generated and used.	Name
DELETE_ CHROM	Deletes the named chromatogram. If "*" is used as the name then the current (default) chromatogram is deleted.	Chromatogram name
OPEN_ CHROM	Opens the specified chromatogram from the specified file.	File name Chromatogram name
RENAME_ CHROM	Renames the specified chromatogram. If "*" is used as *From* then the current (default) chromatogram is used.	From chromatogram name To chromatogram name
RESTORE_ DESTINATION _CHROM	Resets the destination for the subsequent curve and peak table operations to the default chromatogram. Used in pair with the SET_DESTINATION_CHROM instruction.	
SAVE_ CHROM	Saves the specified chromatogram in the specified file. If "*" is used for Name then the current (default) chromatogram is saved. If "*" is used for the ToFile parameter the default file is used.	Name To file
SET_ DESTINATION _CHROM	Opens the named chromatogram as destination for the subsequent curve and peak operations. Used in pair with the RESTORE_DESTINATION_CHROM instruction.	Chromatogram name

C.4.6 Other

BASE	Sets the x-axis base in which the following calculations will be done in. If the value of x-axis base is DEFAULT then the default base is used (usually the base the method was run in). This instruction should be the first in the evaluation procedure otherwise it will have no effect at all.	X-axis base
COMMENT	Inserts a comment below the marked instruction	Comment text
ENDLOOP	Marks the end of a LOOP statement.	
LOOP	The instructions between this statement and the ENDLOOP statement are repeated n times. It is possible to have loops within loops as long as the number of LOOP statements matches the number of ENDLOOP statements.	n Number of loops
REPORT	Prints a report with the specified named report layout and title. If Title is "*" then the title in the report layout is used. If ReportLayout is "*" then a default layout is used.	Report layout Report title
RUN_ PROGRAM	Starts a program as a separate process. The Program name string contains the program name and parameters to start it with.	Program name

D. Feedback tuning

This appendix describes the principles of PID feedback tuning of pump flow rate. UNICORN OS process control software provides a graphical interface for feedback tuning.

Feedback control is aimed at eliminating discrepancies between the actual value and the requested value (in this case flow rate). OligoPilot II and OligoProcess use the measured flow rate to control overall pump speed and the measured conductivity to control the relative speeds of pumps A and B in gradient formation.

PID tuning uses three parameters to determine feedback control:

- •The P parameter reduces the effect of an error but does not completely eliminate it. A simple P-regulator results in a stable discrepancy between actual and requested flow rate (a *stationary error*).

- •The I parameter eliminates the stationary error, but results in a slight instability leading to oscillations in the actual flow. I can have values between 0 and ∞, where smaller values have greater effect and a value of ∞ has no effect. (The value ∞ is set as 9999 in UNICORN OS).

- In certain cases, the D parameter can reduce the oscillations introduced by a PI-regulator. D can have values between 0 and ∞, where larger values have greater effect and a value of 0 has no effect. Most often, a simple PI-regulator is preferable for control of flow rate, and BioProcess System is configured by default with the D parameter set to zero.

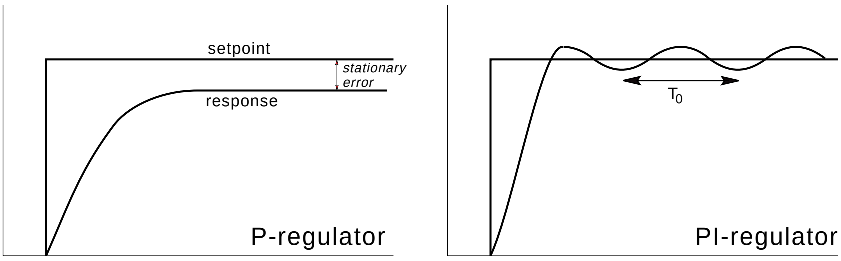


Figure D-1. *A simple P-regulator (left) gives a stationary error. A PI-regulator (right) eliminates the stationary error but introduces stable oscillations in the response.*

Tuning a feedback control system in practice is largely a matter of trial and error. The following recommendations summarize the Ziegler-Nichols method for finding suitable PID-values (small empirical adjustments in the suggested values may be required for optimal feedback control).

D.1 Flow rate tuning

1. Select **System:Tune** in System control. Select **Flow** for **Feedback Control**. Open the valves and start the pump with the column in-line.

2. Set P=0.05, I=9999 and D=0. Set the flow rate in **Setpoint**. Press **New parameters** and the new values will apply.

3. Note the response. Increase the value of P until the actual flow rate oscillates with a constant period and amplitude.

   Note: When changing to new PID values, set the new values and flow rate and press **New parameters**. The flow rate must be changed in **Setpoint** every time the PID values are changed.

   When the oscillation is satisfactory, note the P value (P_0) and the oscillation period in seconds (T_0).

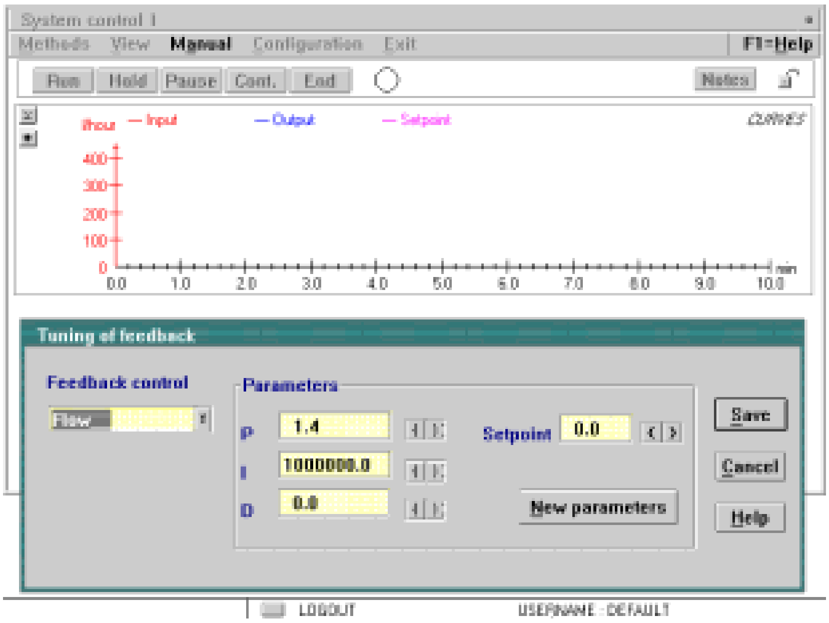
4. Calculate suggested PID values for the required regulator type from the table below.

Regulator type	P	I	D
P	0.5 * P_0		
PI	0.45 * P_0	0.83 * T_0	
PID	0.6 * P_0	0.5 * T_0	0.125 * T_0

5. Adjust the PID parameters from these suggested starting values until the feedback behaviour is satisfactory.

6. When satisfied, press **Save** to save the PID parameter settings.

During tuning adjustments, UNICORN OS displays the effects of the current parameters graphically. The **Output** signal is the signal to the pump. The **Input** signal is the actual flow rate.


System control 1
Methods View Manual Configuration Exit F1=Help
Run Hold Pause Cont. End Notes
l/hour — Input — Output — Setpoint CURVES
400
300
200
100
0
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 min
Tuning of feedback
Save
Cancel
Help
New parameters
LOGOUT
USERNAME : DEFAULT

Feedback control: Flow

Parameters

P: 1.4

I: 1000000.0

D: 0.0

Setpoint: 0.0

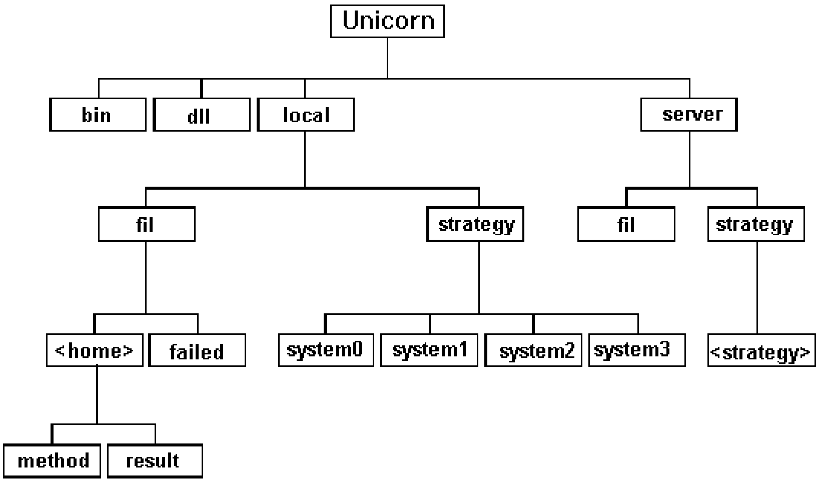
Figure D-2. *PID-tuning in UNICORN OS.*

D.2 Gradient tuning

Tune gradient settings in the same way as flow rate settings, with the exception that the **Setpoint** parameter is %B. The **Input** signal is the actual eluent concentration (%B) as determined by the conductivity.

E. File organisation

This appendix documents the file structure in a UNICORN OS installation.



E.1 Stand-alone installations

In a stand-alone installation, the entire directory structure resides on the local hard disk. System and user definition files and system strategies are duplicated in the `local` and `server` directories.

E.2 Network installations

E.2.1 Local and remote computers

The `bin`, `dll` and `local` directory structures are installed on each computer.

E.2.2 Network server

The `server` directory structure is installed on the network server. The `local\fil` directory structure is also installed if home directories are created on the server disk.

System and user definition files and system strategies are copied automatically from the network server to each station, so that local stations can be used as stand-alone systems in the event of a network communication failure.

Directory	*Description*
UNICORN	The main UNICORN OS directory.
– bin	Executable modules.
– dll	Device drivers and dynamic linked library (dll) modules.
– local	Holds a copy of the global system definitions (system.tab). Not available on remote computers.
– – fil	Holds a copy of the files containing user definitions (user20.mpm). Not available on remote computers.
– – – *<home>*	Home directories for users in the system. There may be any number of home directories in the system. In network installations, home directories may be created on the server disk as well as on local disks.
– – – – method	Holds method files, sequence directories and user-created method directories.
– – – – result	Holds result files, scouting result directories and user-created result directories.
– – – failed	Holds result files in the event of network communication failure.
– – strategy	Holds subdirectories for each system physically connected to the local computer.
– – – system0 – – – system1 – – – system2 – – – system3	System information for systems physically connected to the local computer. Holds a copy of the strategy, flow scheme and templates, and files for the system settings and system audit trail. Directory `system0` corresponds to installed **System 1**. Directories for systems that are not installed are empty.

– server	Holds the original file for system definitions (system.tab). This file is copied to the `local` directory.
– – fil	Holds the original files for user definitions (users20.mpm) and global procedures (globproc.gpl), global report formats (globproc.grf). The user definition file is copied to the `local\fil` directory.
– – strategy	Holds the directory structure for installed strategies.
– – – <*strategy*>	Holds the original files for installed strategies. There is one <*`strategy`*> directory for each installed strategy. Each <*`strategy`*> directory also holds the corresponding template methods (if installed). Strategy files are copied to the `local\strategy` directory according to the systems installed on the local station.

F. Troubleshooting

F.1 Start-up problems

F.1.1 Warning signal from computer

If the computer “beeps” at start-up (after the OS/2 desktop is displayed), this may indicate that you have a local system started in STARTUP.CMD that cannot be initiated. Either edit STARTUP.CMD (see Section 10.7) and reboot the computer to eliminate the system start-up, or define the system in **Administration:System** in the Main menu (see Section 12.1).

F.1.2 UNICORN OS fails to start

Start-up parameters are incorrectly set or UNICORN OS files are damaged or missing.

Check the OS/2 CONFIG.SYS file in the root directory of the boot drive. The following statements are required:

```
...
LIBPATH = ...;drive:\UNICORN\DLL;
...
SET DPRADR = 10
SET DPRVEC = 5
DEVICE = drive:\UNICORN\DLL\DPRCTL.SYS
```

where `drive` is the letter of the drive where UNICORN OS is installed.

Check UNICORN OS program settings. Click on the icon for UNICORN OS with the *right-hand* mouse button on a two-button mouse (the middle button on a three-button mouse) to open the icon menu. Click on the arrow to the right of **Open** and choose **Settings**. The following settings are required:

Path and file name: drive:\UNICORN\BIN\MINIT.EXE
Working directory: drive:\UNICORN\BIN

Both the CONFIG.SYS and UNICORN OS icon settings are set when UNICORN OS is installed. You should not normally need to change these settings. If the settings appear to be correct and UNICORN OS still fails to start, try re-installing UNICORN OS.

F.1.3 OS/2 cannot create swap file

Depending on the amount of memory in your computer, UNICORN OS may require several megabytes free disk space to start. If OS/2 reports that the swap file cannot be created, delete unwanted files from the hard disk to create more free space. About 30 Mb free space is required for the swap file.

UNICORN OS will issue a warning at start-up if the amount of free disk space is limited.

F.2 Login problems

F.2.1 Unable to log in to UNICORN OS

Choose your username from the list and enter your password. If you have forgotten your password, ask the system administrator for a new one.

If you cannot log in using your correct username and password, the USERS20.MPM file in the \UNICORN\SERVER\FIL directory may be corrupt. Restore the file from the latest back-up copy or reinstall the default user (see Section 11.4.2).

If users are not available on a remote station in a network installation (the user list in the login dialogue box is empty), make sure that the computer is logged in to the network before starting UNICORN OS. A remote station accesses the user list directly from the network server.

If the user list on a local station in a network installation is not up to date, make sure that the computer is logged in to the network before starting UNICORN OS. The user list is stored locally on a local station, and is updated automatically from the network server if the computer is logged in to the network.

F.2.2 Error message “Strategy file error”

If you receive the error message “Strategy file error. Can’t load strategy” in a stand-alone installation, the strategy file is probably corrupt. Reinstall the strategy as described in Section 11.4.2.

In a network installation, the error may appear if you try to create a method for a system not physically connected to the computer. Make sure that the computer is logged in to the network before UNICORN OS is started so that the strategy file on the server disk is accessible.

F.3 UNICORN OS access problems

F.3.1 Unable to access certain UNICORN OS functions

UNICORN OS functions to which you do not have access appear grey in the menu and cannot be used. Your user profile is determined by the system administrator from **Administration:User setup** in the Main menu.

F.3.2 Connections are not available

Check the connection between the PC and the synthesis system. Check that the power to the synthesis system is turned on. If the connection appears to be correct and the power is turned on, switch off the synthesis system and quit UNICORN OS. Shut down and switch off the computer, then restart the entire system.

If a system is not available when you attempt to establish a connection, check that you have access rights to the system. Access rights are not automatically assigned for a newly defined system.

If you receive the error message “Cannot connect to system ...” in a network installation, check:

••that the local computer to which the system is connected is turned on and logged in to the network.

••that the computer from which you are trying to establish a connection is logged in to the network.

••that the limit of 8 connections to the system has not been exceeded.

If you can establish a connection but cannot control the system (the manual menu commands in System control are grey), check that no other user has a control mode connection, and that you have sufficient access rights to control the system manually.

F.4 Method and run problems

F.4.1 Cannot switch from control to view mode

If you are unable to switch from control mode to view mode for a connection, you may be running a scouting method or a sequence. These functions require a control mode connection in order to start subsequent cycles correctly.

F.4.2 Monitor signals do not appear in the System control Curves panel

For monitor signals to be displayed in System control, they must be set to **STORE ON** in system settings.

Signals for which **STORE ON** is set can be chosen from the **View:Curve contents** dialogue box for display in the curves panel.

F.4.3 Error message “Couldn’t create result file...”

If you receive an error message “Couldn’t create result file... Destination path could not be found” at the end of a method, the local computer was unable to access the directory specified in the result file path. This may arise if the specified directory is on the network server and network communication has been lost (see Section 9.3.1). The result file is saved in the FAILED directory on the local station.

F.5 Evaluation problems

F.5.1 Incorrect date and time

The date and time recorded in the result file are taken from the PC system clock setting. If these are not correct, check the system clock setting.

F.5.2 Evaluation procedure aborts

Instructions in an evaluation procedure address curves by identification number irrespective of curve names. Make sure that the curves processed when the procedure is executed are compatible with those processed when it was recorded. An evaluation procedure aborts if you try to store resulting curves at the position of an original raw data curve.

F.6 Other problems

If system errors arise during a run, these are displayed in a dialogue box with a description of the error. If you do not understand the error description or are unable to correct the error situation, contact technical service.

Note: Some information, warning and error displays arise from OS/2, not from UNICORN OS. If you have installed a different language version of OS/2, these displays will be in the OS/2 language. UNICORN OS displays will however still be in English.

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